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WATER NUTRIENTS NUMBER 2

Raymond J. Lishka, et al

Environmental Control Administration
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WATER NUTRIENTS NO. 2

Study Number 36

Report of a Study Conducted by

ANALYTICAL REFERENCE SERVICE

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Environmental Health Service
Cincinnati, Ohio 45213
1970

FOREWORD

The Analytical Reference Service (ARS) is conducted by the Bureau of Water Hygiene of the Environmental Control Administration to evaluate laboratory methods in the environmental field. Cooperative studies by member organizations, who analyze identical samples and critically review methodology, provide the mechanism for:

> Evaluation of analytical procedures, including precision and accuracy, by comparison of the procedures and results reported by participating laboratories.
>
> Exchange of information regarding method characteristics.
>
> Improvement or replacement of existing methods by development of more accurate procedures, and development of new methodology for determination of new pollution compounds.

Samples are designed to contain measured amounts of selected constituents. Decisions as to qualitative makeup are made by the membership, consultants, and the ARS staff. Notice of each study is sent to the entire membership. To those who desire to participate, a portion of the study sample is sent, along with data forms for reporting numerical values, a critique of the procedures used, comments on modifications, sources of error, difficulties encountered, or other pertinent factors. The results and comments received are compiled, and a report of each study is prepared.

Now primarily directed toward examination of water, in the past studies have included methods for analysis of air, milk, and food. Some studies are periodically repeated for the advantage of new members, the evaluation of new methods, or the reevaluation of existing methods.

The selection of studies is guided by requests from standard methods committees and the responses to questionnaires periodically circulated among the membership, which now includes 299 Federal, state, and municipal agencies; industries; universities; consulting firms; and foreign agencies.

STUDIES COMPLETED AND REPORTED

Water-Minerals	Calcium, magnesium, hardness, sulfate, chloride, alkalinity, nitrite, nitrate, sodium, and potassium. Studies completed in 1956, 1958, and 1961.
Water-Metals	Lead, copper, cadmium, aluminum, chromium, iron, manganese, and zinc. Studies completed in 1957 and 1962. These same metals plus silver. Study completed in 1965. Except for the substitution of magnesium for aluminum, these same metals were analyzed by atomic absorption in 1967. Copper, manganese, and aluminum in the presence and absence of interferences. Study completed in 1969.
Water-Fluoride	Fluoride in the presence and absence of interferences, with and without distillation by a specified procedure. Studies completed in 1958 and 1961. Fluoride by ion-exchange and fluoride electrode. Study completed in 1969.
Water-Radioactivity	Gross beta activity. Studies completed in 1959 and 1961. Gross beta and strontium-90 activity. Study completed in 1963.
Water-Surfactant	Surfactant in various waters. Studies completed in 1959, 1963, and 1968.
Water-Oxygen Demand	Biochemical oxygen demand and chemical oxygen demand. Study completed in 1960. Chemical oxygen demand. Study completed in 1965.
Water-Trace Elements	Arsenic, boron, selenium, and beryllium. Study completed in 1962. These same metals plus vanadium. Study completed in 1966.
Freshwater Plankton	Evaluation of the precision and accuracy obtainable by the use of various methods of plankton counting and identification. Study completed in 1964.

Water-Nutrients	Silicate, phosphate, ammonia nitrogen, organic nitrogen, and nitrate nitrogen. Study completed in 1966. Ammonia nitrogen, nitrate nitrogen, and ortho, poly, and organic phosphate. Study completed in 1969.
Water-Phenols	Phenol and 2,4-dichlorophenol in water by two specified methods. Study completed in 1966.
Water-Cyanides	Potassium cyanide and potassium ferricyanide in water by two specified methods. Study completed in 1967.
Water-Chlorine	Free and combined chlorine by nine different methods. Study completed in 1969.
Air-Inorganics	Chloride, sulfate, fluoride, and nitrate in aqueous solution and on glass-fiber, high-volume filter mats. Study completed in 1958.
Air-Lead	Filter paper tape impregnated with lead. Study completed in 1961.
Air-Particulates	Microscopic identification of some common atmospheric particulates. Study completed in 1964.
Air-Sulfur Dioxide	Sulfur dioxide in air by a specified method. Study completed in 1963.
Water-Pesticides	Lindane, heptachlor epoxide, DDE, and dieldrin in water. Study completed in 1965. Lindane, heptachlor, aldrin, heptachlor epoxide, p,p'-DDE, dieldrin, endrin, o,p'-DDT, p,p'-DDT, and methoxychlor in water. Study completed in 1968.
Food-Pesticides	DDT in milk. Study completed in 1962. Lindane, heptachlor epoxide, DDE, and dieldrin in milk. Study completed in 1965.

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PREFACE

Phosphorus occurs in natural waters and in waste water as orthophosphate, as condensed phosphates (pyro, meta, and poly), and as organically bound phosphates. These forms of phosphate may occur in the soluble form, as inorganic or organic particulates, or in the bodies of aquatic organisms. Inasmuch as phosphorus is an element which is essential to the growth of organisms, the discharge of phosphorus containing wastes into a receiving water may stimulate the growth of photosynthetic microorganisms. On the other hand, phosphate is also added frequently to domestic and industrial waters in various forms to overcome many problems associated with water hardness. Phosphorus, therefore, is important in any water treatment operation. Unfortunately, the methods presented in the 12th edition of Standard Methods for the Examination of Water and Wastewater are inadequate since no provision is made for the determination of organic phosphate or for the differentiation of soluble and insoluble phosphate. In an effort to rectify this situation the procedures proposed for the 13th edition have been completely rewritten and are those evaluated by this study.

Likewise, nitrates generally occur in trace quantities in surface water supplies but may attain larger concentrations owing to agricultural drainage, discharge of treated or raw sewage, and certain industrial wastes. As with phosphates, these increases may stimulate the growth of nuisance organisms. As a result of leaching from the soil, nitrates may also reach large concentrations in some ground waters. Since, in excess amounts, it contributes to the illness known as infant methemoglobinemia, a limit of 10 milligrams of nitrate nitrogen per liter has been imposed on drinking water. The three methods for nitrate measurement included in this study are those proposed for the 13th edition. Two of these are new, but one of these was previously partially evaluated.

Ammonia when encountered in raw surface waters is sometimes accepted as chemical evidence of sanitary pollution since it is a product of microbiological activity. It may occur in ground water, however, as a result of natural reduction processes. Where the free residual chlorination process is employed, the presence of ammonia in the water supply may require the addition of large amounts of chlorine in order to produce a free chlorine residual. On the other hand, where combined residual chlorination is practiced, the addition of ammonia is necessary; hence the need for a good method for ammonia determination. In a previous study, only the nesslerization procedure was found satisfactory. In this study the newly proposed "phenate" method is evaluated.

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ACKNOWLEDGMENTS

This study was undertaken at the suggestion of Michael Taras, Chairman of the Joint Editorial Board for Standard Methods for the Examination of Water and Wastewater, in order to evaluate some new methods for the determination of ammonia, phosphates, and nitrates, which have been proposed for inclusion in the next edition.

Dr. David Jenkins, Associate Professor of Sanitary Engineering, University of California, Berkeley, and chairman of the Standard Methods Subcommittee on Nutrients was consulted in regard to the composition of the proposed samples and reviewed this report before publication.

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Atomic Energy Authority, Didcot, Berks, England
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California Department of Water Resources, Sacramento
California Water Service Company, San Jose
Carnegie-Mellon University, Pittsburgh, Pennsylvania
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Central Water Filtration Plant, Chicago, Illinois
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Johns-Manville Research & Engineering Center, Manville, New Jersey
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Long Beach Water Department, California
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Maryland State Department of Health, Baltimore
Mekoroth Water Company, Bnei-Brak, Israel
Metropolitan Water District of Southern California, La Verne
Metropolitan Water, Sewerage and Drainage Board, Sydney, Australia
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Minneapolis Water Department, Minnesota
Municipal Laboratories, Ontario, Canada
Nassau County Department of Health, Hempstead, New York
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New Jersey State Department of Health, Trenton

New Mexico Department of Public Health, Albuquerque
New York State Department of Health, Albany
North Carolina Department of Water & Air Resources, Raleigh
North Jersey District Water Supply Commission, Wanaque, New Jersey
Ontario Water Resources Commission, Canada
Oregon State Board of Health, Portland
Pan American World Airways, Patrick AFB, Florida
Pennsylvania Department of Health, Harrisburg
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Regional Environmental Health Laboratory, Kelly AFB, Texas
Regional Environmental Health Laboratory, McClellan AFB, California
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Sandia Corporation, Albuquerque, New Mexico
Seattle Water Department, Washington
South Carolina Pollution Control Authority, Columbia
Springwells Filtration Plant, Dearborn, Michigan
State Department of Public Health, Los Angeles, California
St. Louis County Water Company, Missouri
Tennessee Valley Authority, Chattanooga, Tennessee
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USDI, FWPCA, Ohio River Basin Project, Evansville, Indiana
USDI, FWPCA, Pomona, California
USDI, FWPCA, Wheeling Field Station, West Virginia
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USDI, Geological Survey, Harrisburg, Pennsylvania
USDI, Geological Survey, Little Rock, Arkansas
University of Alberta, Edmonton, Canada
University of Beograd, Yugoslavia
University of North Carolina, Chapel Hill
Utah State Division of Health, Salt Lake City
Virginia State Water Control Board, Richmond
Washington State Department of Health, Seattle
Washington State University, Pullman

ABSTRACT

In this report the results obtained on analysis of three water samples by selected methods for ammonia nitrogen, nitrate nitrogen, orthophosphate, polyphosphate, and total phosphate are compared and evaluated.

Nesslerization is more accurate and precise than the phenate method for the measurement of ammonia nitrogen. For nitrate nitrogen, the preferred method is the modified brucine (Jenkins and Medsker) method, although, if suitable reagents become available, the chromotropic acid and cadmium reduction methods would be equally suitable. Hydrazine reduction used with the AutoAnalyzer also appears satisfactory. For phosphate measurement, the stannous chloride method for orthophosphate, preceded by mild acid hydrolysis for condensed phosphates, or digestion with sulfuric-nitric acid for total phosphate, is the most generally satisfactory procedure.

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DESIGN OF THE STUDY

In this study, three samples containing ammonia, nitrate, orthophosphate, polyphosphate, and organic phosphate were provided. In order to ensure stability these samples were adjusted to pH 8.0 to prevent hydrolysis of the polyphosphate. Twenty mg of mercury per liter (as mercuric chloride) was added as a preservative, and the solutions were prepared as concentrates. Each participant was instructed to dilute before the analysis 5 ml of each concentrated sample to a liter with a good quality distilled water. When the samples were diluted as instructed their compositions were as indicated in Table 1. The participants were not asked to analyze the samples for sodium chloride, which was added as a possible interference in the nitrate determination.

The purpose of this study was to evaluate the new methods for the determination of the above-mentioned elements, which have been proposed for inclusion in the 13th edition of Standard Methods for the Examination of Water and Wastewater. Copies of these methods (see appendices) were sent with the samples. They include the phenate method for ammonia nitrogen; the chromotropic acid, modified brucine, and the cadmium reduction methods for nitrate nitrogen; the stannous chloride and vanadomolybdic acid methods for ortho-phosphate; a dilute acid hydrolysis procedure for condensed (poly) phosphates; and digestion with perchloric, sulfuric-nitric acid or by persulfate oxidation to determine total (organic) phosphate. The latter phosphate methods were designed in particular for the analysis of natural waters, but the intent was to make them applicable

Table 1. COMPOSITION OF SAMPLES

Compound	Nutrient	Sample 1	Sample 2	Sample 3
KH_2PO_4	Orthophosphate, mg P/liter	0.10	0.60	7.00
$(NaPO_3)_6$	Polyphosphate, mg P/liter	0.08	0.30	3.00
Adenylic Acid	Organic Phosphate, mg P/liter	0.03	0.09	0.23
Sum of above	Total Phosphate, mg P/liter	0.21	0.99	10.23
NH_4Cl	Ammonia, mg N/liter	1.50	0.80	0.20
KNO_3	Nitrate, mg N/liter	0.50	5.00	0.05
NaCl	Cl^- interference, mg/liter	400	400	400

also to treated waters. For those interested only in the analysis of phosphate-treated waters, a bismuth-catalyzed ammonium molybdate method was also provided.

In order to obtain sufficient data on each of these methods, each participant was asked to analyze each of the samples by at least two of the three provided methods for nitrate, orthophosphate, polyphosphate, and total phosphate. Since the samples contained only soluble phosphate the participants were told that it would not be necessary to attempt to differentiate between total (particulate plus soluble) and total filterable (soluble) phosphate.

TREATMENT OF DATA

As the results of analysis were received from each participant, the data were reviewed, and in the case of a suspected mathematical error, the reporting laboratory was asked to check the calculations and to submit a corrected value if an error was found.

The data were then coded and analyzed by computer for normality of distribution and subsequent rejection of outliers that were nonrepresentative because of errors in calculation, dilution, or other indeterminate factors. The results for each method were also plotted on normal probability paper as a visual check on normality of distribution and the presence of outliers. After verification of normality of distribution and the rejection of outliers, the data were statistically analyzed by computer for precision and accuracy (see Appendices G and H).

Bar graphs are included in the text to provide a pictorial display of the data. Rejected values are indicated by broken bars, and values are given for bars off the scale of the chart.

The individual results tabulated in Appendix E are catalogued according to the method numbers listed in the bibliography of this report under ammonia nitrogen, nitrate nitrogen, orthophosphate, polyphosphate, or total phosphate.

DISCUSSION OF RESULTS

AMMONIA NITROGEN (Table 2, Figures 1-6)

The phenate method was used by 72 participants and, rather surprisingly, produced the poorest accuracy in the sample with the largest concentration of ammonia. For sample 1, containing 1.50 mg/liter, the relative error of 10.0 percent and relative standard deviation of 26.0

Table 2. SUMMARY OF AMMONIA RESULTS

Method	No. results	No. outliers	Mean	Mean error	Std. dev.	Rel. error	Rel. std. dev.	95% tol. limits
			Sample 1, 1.50 mg/liter nitrogen					
Phenate[a]	71	1	1.3507	-0.149	0.3515	10.0	26.0	±0.81
Dist. and phenate	3	0	1.5067	+0.007	0.1102	0.4	7.3	±1.09
Nessler.	8	1	1.5188	+0.019	0.0811	1.2	5.3	±0.30
Dist. and nessler.	6	0	1.4467	-0.053	0.1080	3.6	7.5	±0.48
AutoAnalyzer with phenate	3	0	1.6733	+0.173	0.2969	11.6	17.7	±2.94
			Sample 2, 0.80 mg/liter nitrogen					
Phenate[a]	66	6	0.7877	-0.012	0.1243	1.5	15.8	±0.29
Dist. and phenate	3	0	0.7867	-0.013	0.1305	1.7	16.6	±1.29
Nessler.	9	0	0.7978	-0.002	0.1286	0.3	16.1	±0.45
Dist. and nessler.	6	0	0.7750	-0.025	0.1266	3.1	16.3	±0.56
AutoAnalyzer with phenate	3	0	0.9367	+0.137	0.1850	17.1	19.8	±1.83
			Sample 3, 0.20 mg/liter nitrogen					
Phenate[a]	70	2	0.1951	-0.005	0.0766	2.4	39.2	±0.18
Dist. and phenate	3	0	0.1667	-0.033	0.0252	16.7	15.1	±0.25
Nessler.	9	0	0.2167	+0.017	0.0477	8.3	22.0	±0.17
Dist. and nessler.	5	1	0.2040	+0.004	0.0321	2.0	15.7	±0.16
AutoAnalyzer with phenate	3	0	0.3033	+0.103	0.1274	51.7	42.0	±1.26

[a]Method requested by ARS.

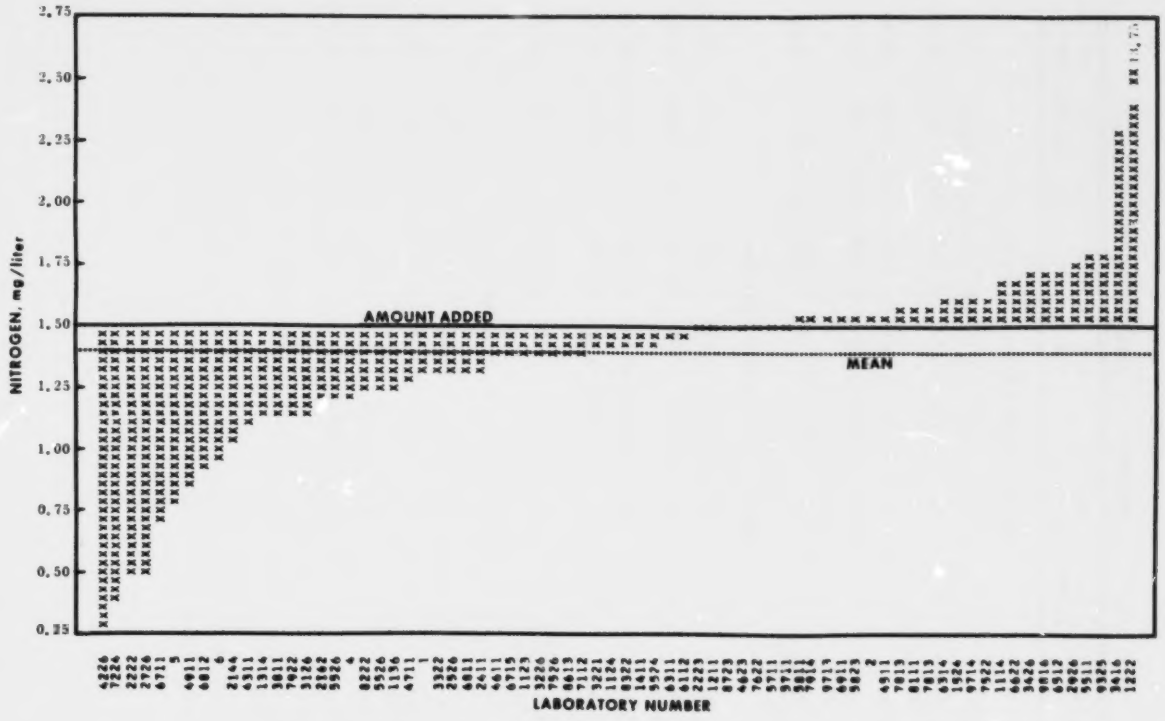

Figure 1. Bar graph for ammonia in sample 1 by the phenate method.

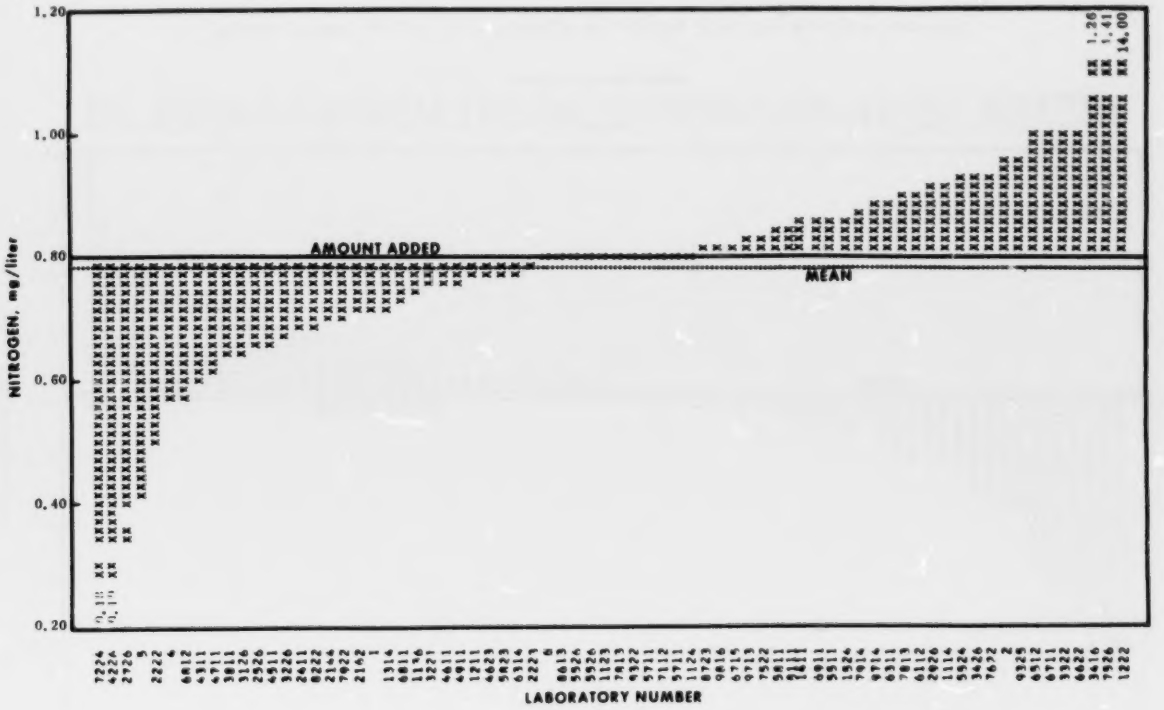

Figure 2. Bar graph for ammonia in sample 2 by the phenate method.

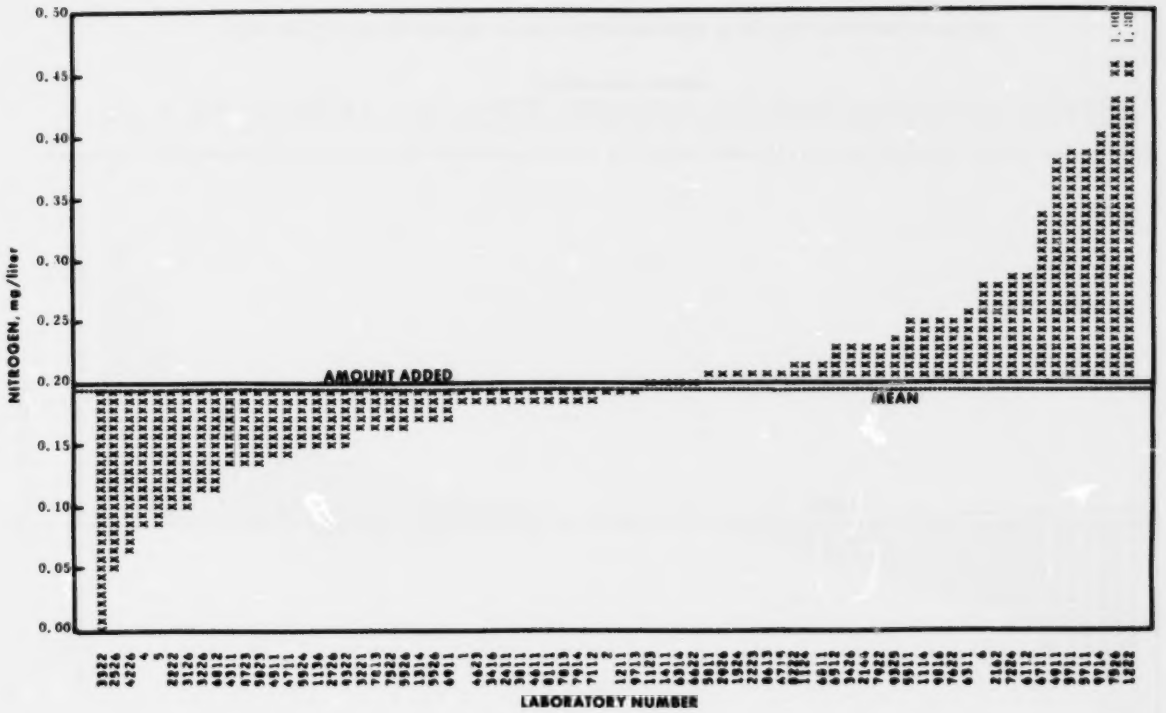

Figure 3. Bar graph for ammonia in sample 3 by the phenate method.

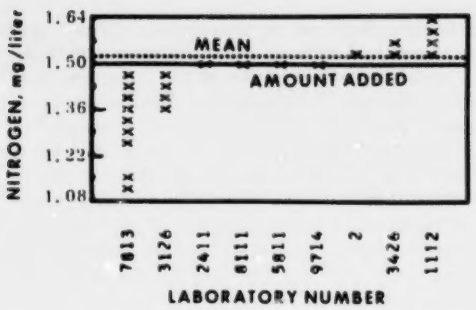

Figure 4. Bar graph for ammonia in sample 1 by nesslerization.

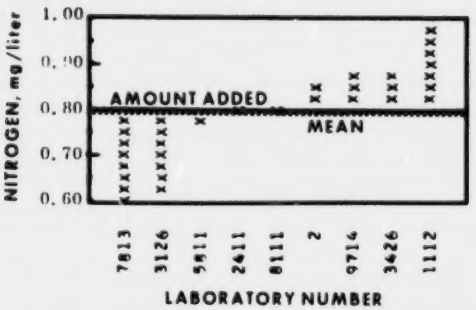

Figure 5. Bar graph for ammonia in sample 2 by nesslerization.

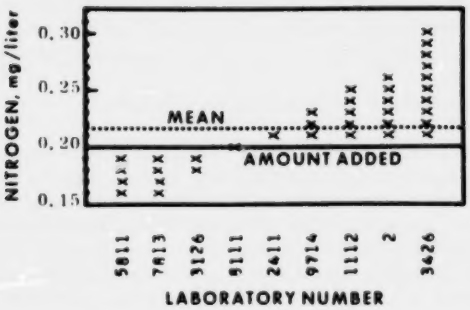

Figure 6. Bar graph for ammonia in sample 3 by nesslerization.

percent indicate unacceptable performance. Three participants that used the phenate method with an AutoAnalyzer produced only slightly better results with a relative error of 11.6 percent and relative standard deviation of 17.7 percent. Although no interferences were present, three participants using the phenate method preceded by distillation produced good results with a relative error of 0.4 percent and relative standard deviation of 7.3 percent. Eight results obtained by nesslerization show the best accuracy and precision, as indicated by a relative error of 1.2 percent and relative standard deviation of 5.3 percent. Six participants who distilled the sample before nesslerization were slightly less accurate and less precise with a relative error of 3.6 percent and relative standard deviation of 7.5 percent.

For sample 2, containing 0.80 mg/liter, the phenate method used with the AutoAnalyzer produced unacceptable results. All the other methods were approximately equal with good accuracy and acceptable precision.

At the smallest concentration of 0.20 mg/liter in sample 3, only the results by distillation followed by nesslerization are acceptable. The phenate and nesslerization methods were fairly accurate but very imprecise, as shown by the respective relative errors of 2.4 percent and 8.3 percent and respective relative standard deviations of 39.2 percent and 22.0 percent. Distillation followed by the phenate method produced fair precision but poor accuracy, as indicated by the relative standard deviation of 15.1 percent and relative error of 16.7 percent. Both accuracy and precision were poor for the AutoAnalyzer with phenate, as shown by the relative error of 51.7 percent and relative standard deviation of 42.0 percent.

At each concentration, nesslerization was superior to the phenate method, but only in sample 1 is this difference statistically significant. When the phenate method was applied to the AutoAnalyzer, the results were unacceptably high and imprecise regardless of concentration.

NITRATE NITROGEN (Table 3, Figures 7-15)

The three methods requested to be used in this study, namely, chromotropic acid, modified brucine (Jenkins and Medsker), and cadmium reduction, all appear very similar in performance at the three concentrations of nitrate provided. The cadmium reduction appears slightly more accurate and precise at the 5.0 mg/liter level (sample 2), but the difference is not statistically significant. All three methods are suitable at 5.0 mg/liter and 0.5 mg/liter, but all show unacceptable precision (relative standard deviations of 61.4 percent, 66.7 percent, and 96.4 percent) at the 0.05 mg/liter level. The cadmium reduction method had significantly poorer accuracy as well (relative error of 47.3 percent versus

Table 3. SUMMARY OF NITRATE RESULTS

Method	No. results	No. outliers	Mean	Mean error	Std. dev.	Rel. error	Rel. std. dev.	95% tol. limits
			Sample 1, 0.50 mg/liter nitrogen					
Chromotropic acid[a]	34	5	0.5176	+0.018	0.0850	3.5	16.4	±0.21
Modified brucine[a]	50	5	0.5028	+0.003	0.0725	0.6	14.4	±0.17
Cadmium reduction[a]	11	1	0.4682	-0.032	0.1198	6.4	25.6	±0.39
AutoAnalyzer with hydrazine reduction	4	0	0.5000	0.000	0.0082	0.0	1.6	±0.05
Brucine	3	0	0.4033	-0.097	0.1343	19.3	33.3	±1.33
			Sample 2, 5.00 mg/liter nitrogen					
Chromotropic acid[a]	36	3	4.8564	-0.144	0.5273	2.9	10.9	±1.31
Modified brucine[a]	50	5	4.7738	-0.226	0.7326	4.5	15.4	±1.74
Cadmium reduction[a]	10	2	4.9480	-0.052	0.4543	1.0	9.2	±1.54
AutoAnalyzer with hydrazine reduction	4	0	5.0450	+0.045	0.1038	0.9	2.1	±0.66
Brucine	3	0	4.0333	-0.967	0.8005	19.3	19.9	±7.94
			Sample 3, 0.05 mg/liter nitrogen					
Chromotropic acid[a]	32	4	0.0563	+0.006	0.0345	12.5	61.4	±0.09
Modified brucine[a]	50	4	0.0462	-0.004	0.0308	7.6	66.7	±0.07
Cadmium reduction[a]	11	0	0.0264	-0.024	0.0254	47.3	96.4	±0.08
AutoAnalyzer with hydrazine reduction	4	0	0.0575	+0.008	0.0096	15.0	16.7	±0.06
Brucine	3	0	0.0233	-0.027	0.0321	53.3	137.8	±0.32

[a]Methods requested by AHS.

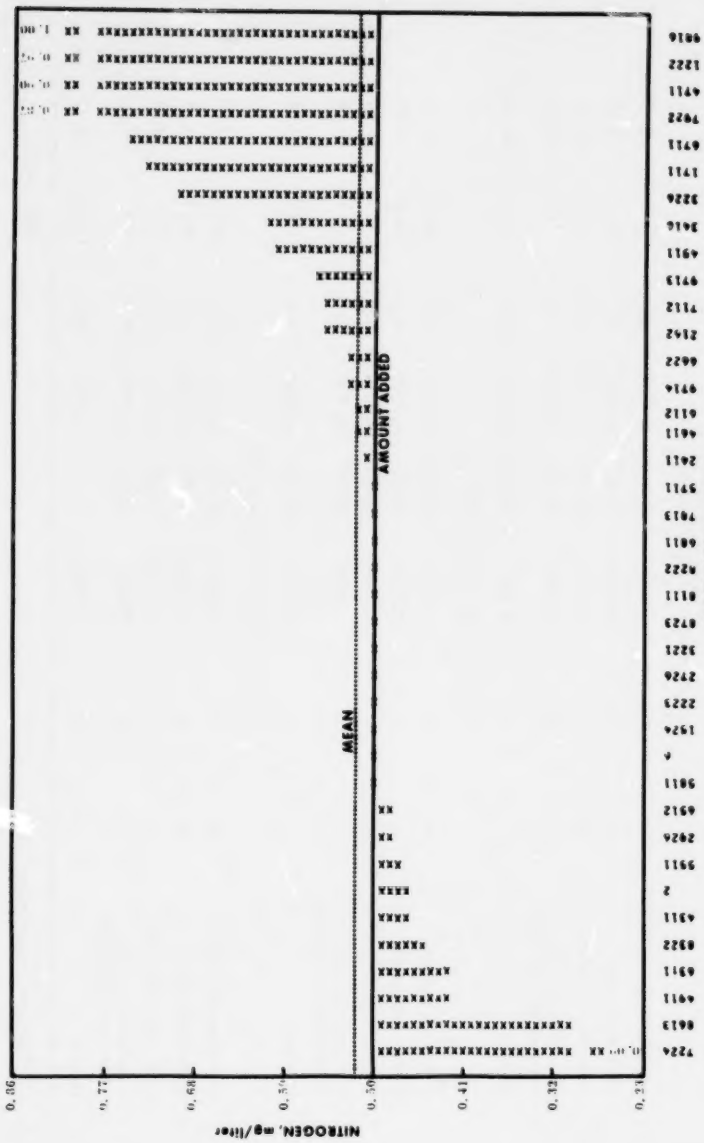

Figure 7. Bar graph for nitrate in sample 1 by chromotropic acid.

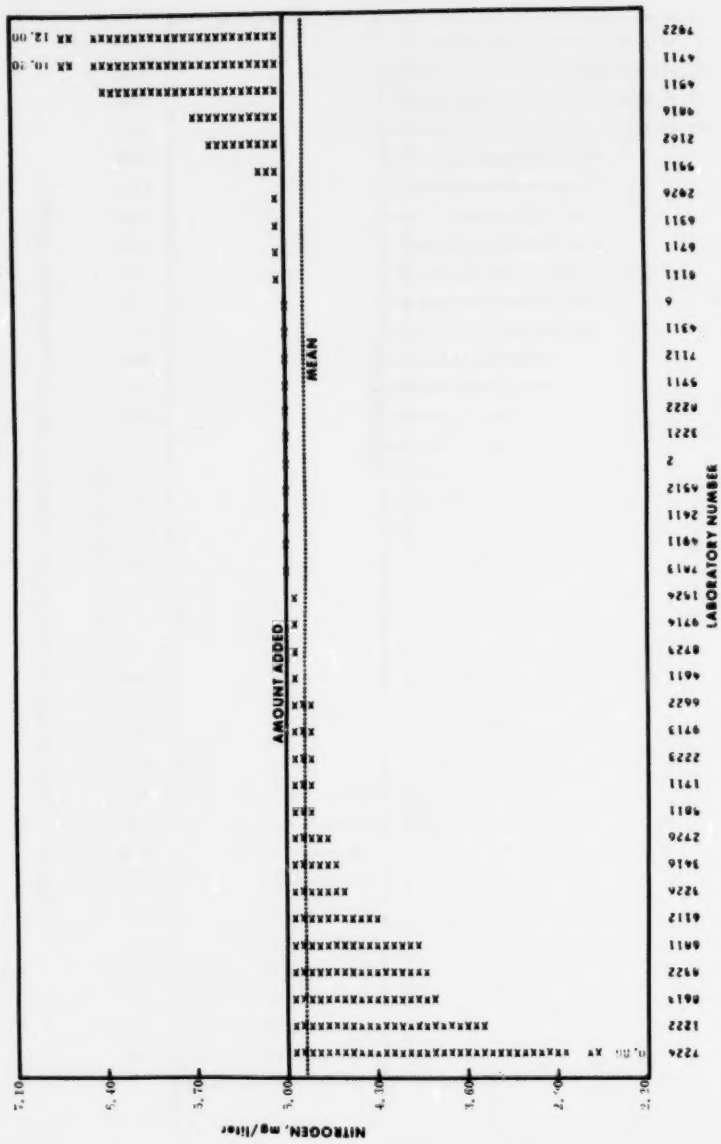

Figure 8. Bar graph for nitrate in sample 2 by chromotropic acid.

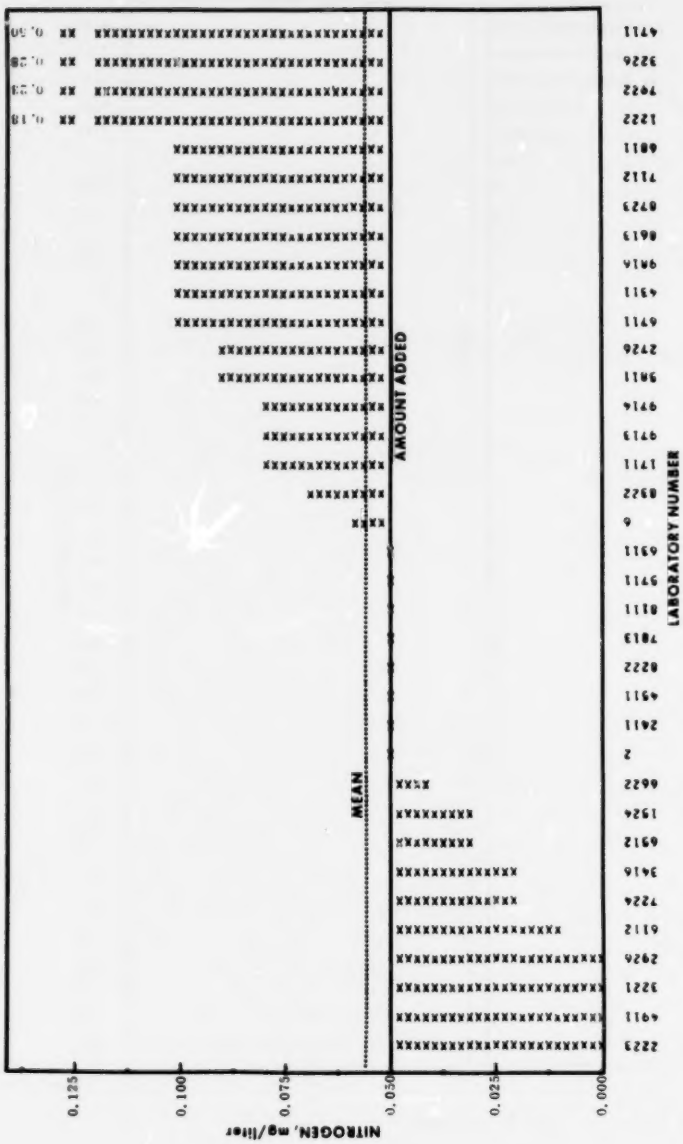

Figure 9. Bar graph for nitrate in sample 3 by chromotropic acid.

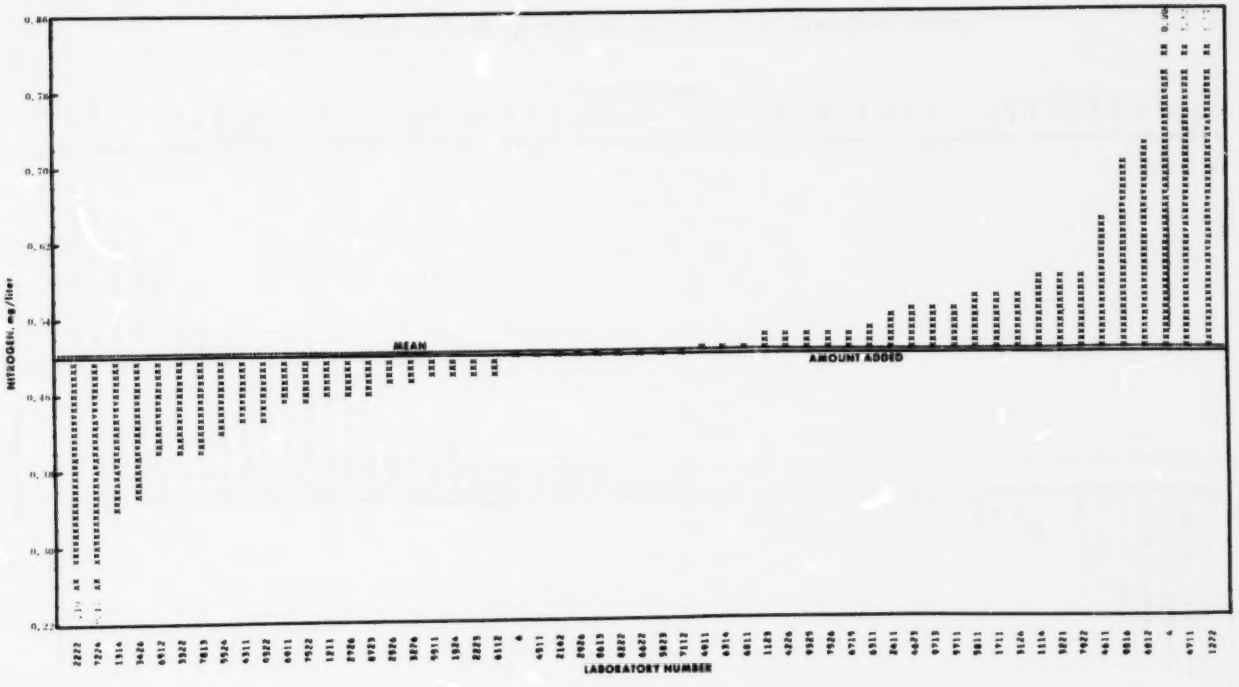

Figure 10. Bar graph for nitrate in sample 1 by modified brucine.

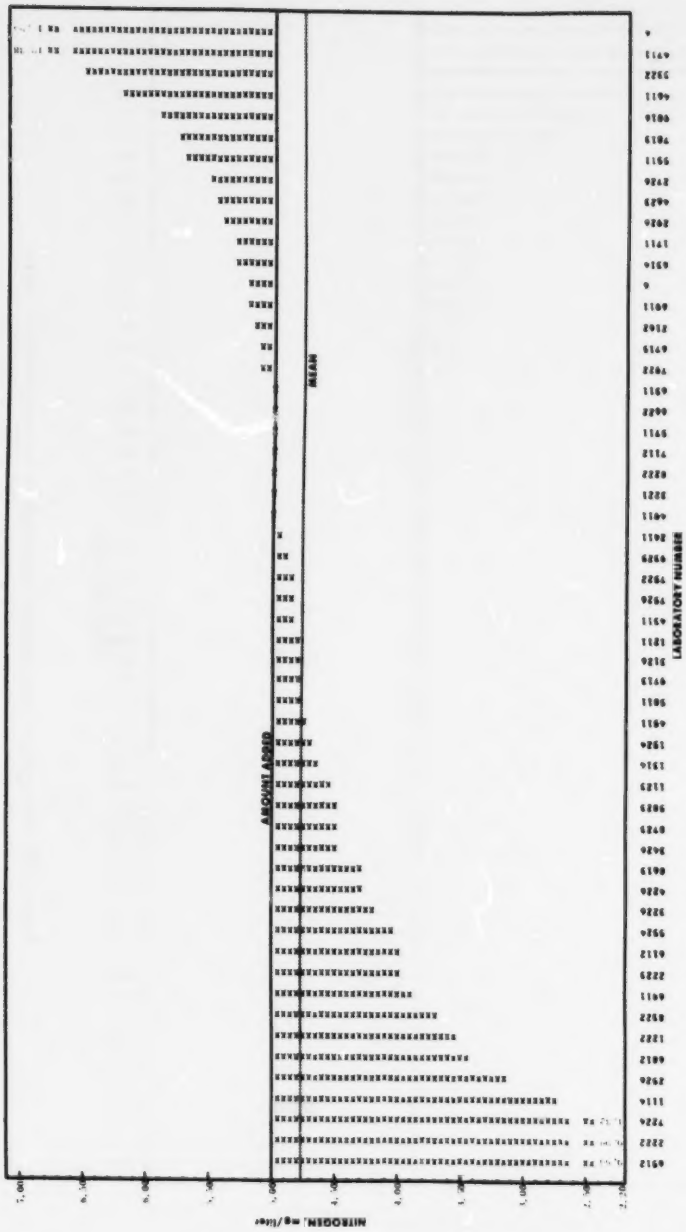

Figure 11. Bar graph for nitrate in sample 2 by modified brucine.

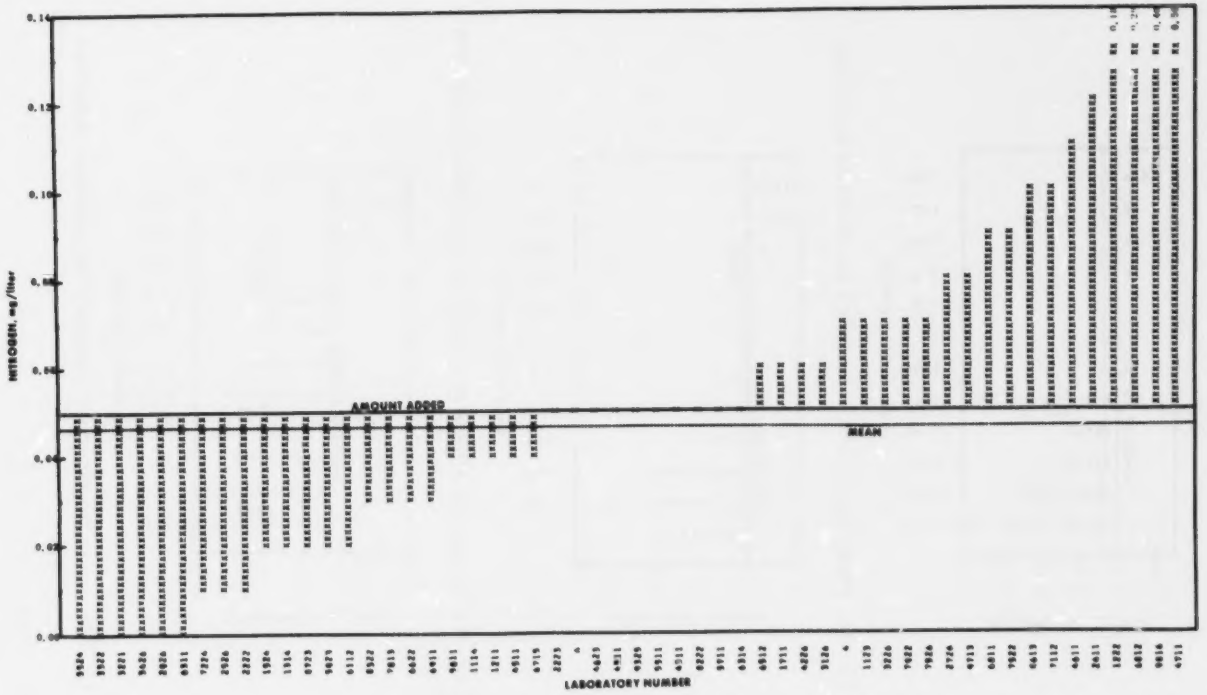

Figure 12. Bar graph for nitrate in sample 3 by modified brucine.

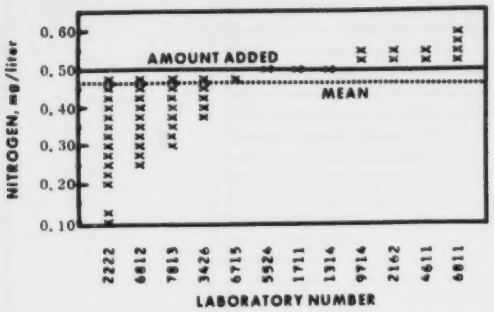

Figure 13. Bar graph for nitrate in sample 1 by cadmium reduction.

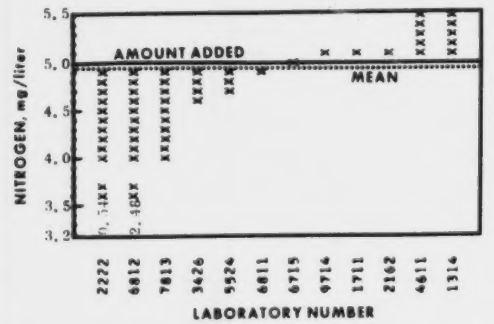

Figure 14. Bar graph for nitrate in sample 2 by cadmium reduction.

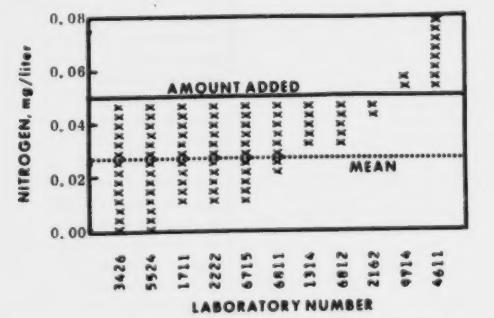

Figure 15. Bar graph for nitrate in sample 3 by cadmium reduction.

12.5 percent and 7.6 percent) for chromotropic acid and modified brucine, respectively.

The AutoAnalyzer used with a hydrazine reduction method by four participants produced excellent accuracy and precision in all three samples. Although four results are too few to draw firm conclusions, the excellent performance is worthy of note.

On the other hand, the poorest accuracy and precision for all three samples was shown by three results obtained with the older brucine procedure from Standard Methods for the Examination of Water and Wastewater, but again there are too few values to make a reliable conclusion. In the previous Water Nutrients No. 1 study it was also found that the modified brucine and chromotropic acid procedures were approximately equal in both precision and accuracy.

ORTHOPHOSPHATE (Table 4, Figures 16-24)

All methods used for orthophosphate measurement produced high results on all three samples, and most of the polyphosphate measurements were low. This arouses the suspicion that a small amount of polyphosphate hydrolyzed to orthophosphate and thereby increased the orthophosphate content. In the ARS laboratory it was found that no hydrolysis of the concentrates occurred but that if the samples were not analyzed immediately after dilution some hydrolysis did occur since the diluted samples were no longer of as high a pH (originally about 8.0). Thus, it would appear that most of the participants did not analyze for phosphates immediately after diluting the samples but, as might be expected, proceeded with the ammonia and nitrate determinations before undertaking the phosphate analysis. The evaluation of the phosphate results with respect to accuracy, therefore, can be only relative between the methods employed. As it turned out, the three methods requested, namely, stannous chloride, vanadomolybdate, and bismuth-catalyzed ammonium molybdate, show no statistically significant difference in accuracy. At 0.10 mg/liter (sample 1), both the stannous chloride and bismuth-catalyzed methods were significantly more precise than the vanadomolybdate method. At 0.60 mg/liter (sample 2), the stannous chloride method was significantly more precise than both of the other methods. At the large concentration of 7.00 mg/liter (sample 3), the bismuth-catalyzed ammonium molybdate method is significantly more precise than the other methods. The stannous chloride method is known to become nonlinear at concentrations greater than 2.0 mg/liter, and it is likely that some of the more erroneous values are the result of failing to dilute the sample before analysis.

Three participants modified the ammonium molybdate procedure by using ascorbic acid as the reducing agent and achieved good accuracy

Table 4. SUMMARY OF ORTHOPHOSPHATE RESULTS

Method	Number of results	Number of outliers	Mean	Mean error	Std. dev.	Rel. error	Rel. std. dev.	95% tol. limits
	Sample 1, 0.10 mg/liter phosphorus							
Stannous Chloride[a]	45	2	0.1287	+0.029	0.0328	28.7	25.5	±0.08
Vanadomolybdate[a]	45	2	0.1216	+0.022	0.0914	21.6	75.2	±0.22
Ammonium molybdate with bismuth[a]	17	0	0.1382	+0.038	0.0396	38.2	28.6	±0.11
Ammonium molybdate with ascorbic acid	3	0	0.1100	+0.010	0.0100	10.0	9.1	±0.10
Murphy and Riley Reagent	4	0	0.1675	+0.068	0.0457	67.5	27.3	±0.29
AutoAnalyzer with stannous chloride	4	0	0.1325	+0.033	0.0457	32.5	34.5	±0.29
	Sample 2, 0.60 mg/liter phosphorus							
Stannous Chloride[a]	44	3	0.6477	+0.048	0.0923	8.0	14.2	±0.22
Vanadomolybdate[a]	43	4	0.6647	+0.065	0.1299	10.8	19.6	±0.31
Ammonium molybdate with bismuth[a]	17	0	0.7006	+0.101	0.1390	16.8	19.8	±0.40
Ammonium molybdate with ascorbic acid	3	0	0.6267	+0.027	0.0252	4.4	4.0	±0.25
Murphy and Riley Reagent	3	1	0.8033	+0.203	0.0153	33.9	1.9	±0.15
AutoAnalyzer with stannous chloride	3	1	0.6033	+0.003	0.0289	0.6	4.8	±0.29
	Sample 3, 7.00 mg/liter phosphorus							
Stannous Chloride[a]	45	2	7.2982	+0.298	0.5543	4.3	7.6	±1.33
Vanadomolybdate[a]	44	3	7.3755	+0.376	0.6361	5.4	8.6	±1.54
Ammonium molybdate with bismuth[a]	12	5	7.2883	+0.288	0.0816	4.1	1.1	±0.26
Ammonium molybdate with ascorbic acid	3	0	7.3433	+0.343	0.3855	4.9	5.2	±3.82
Murphy and Riley Reagent	4	0	7.8900	+0.890	0.4748	12.7	6.0	±3.02
AutoAnalyzer with stannous chloride	4	0	7.9700	+0.970	1.1808	13.9	14.8	±7.52

[a]Methods requested by ARS.

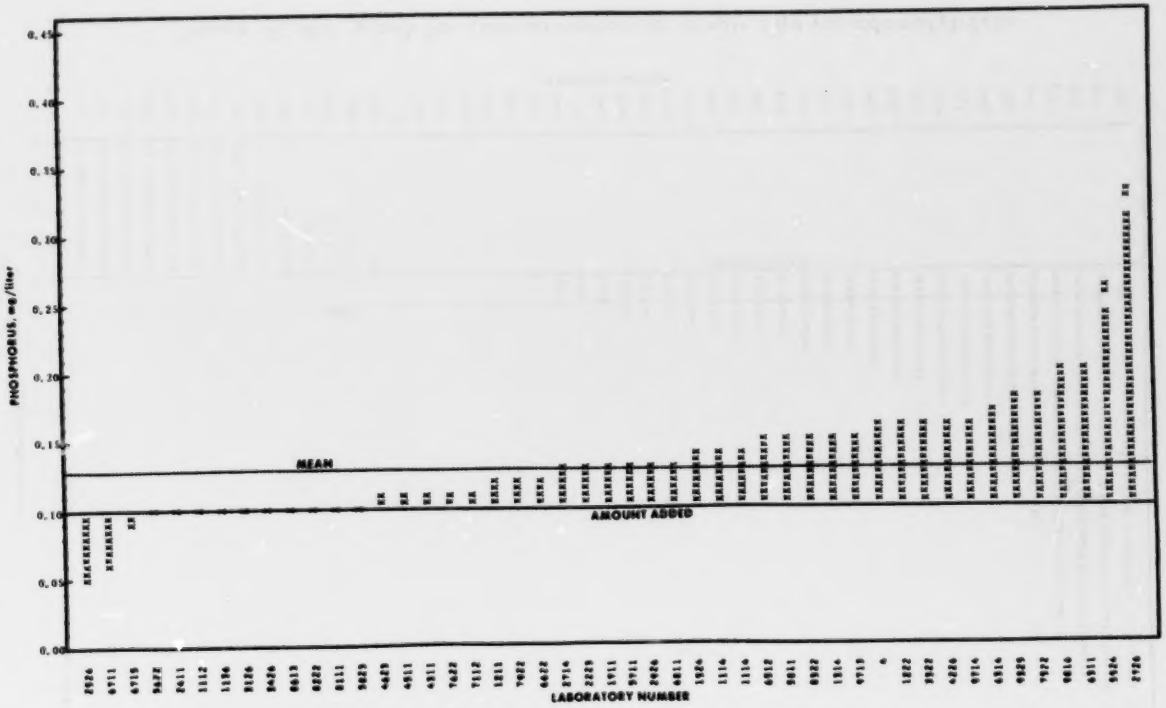

Figure 16. Bar graph for orthophosphate in sample 1 by stannous chloride.

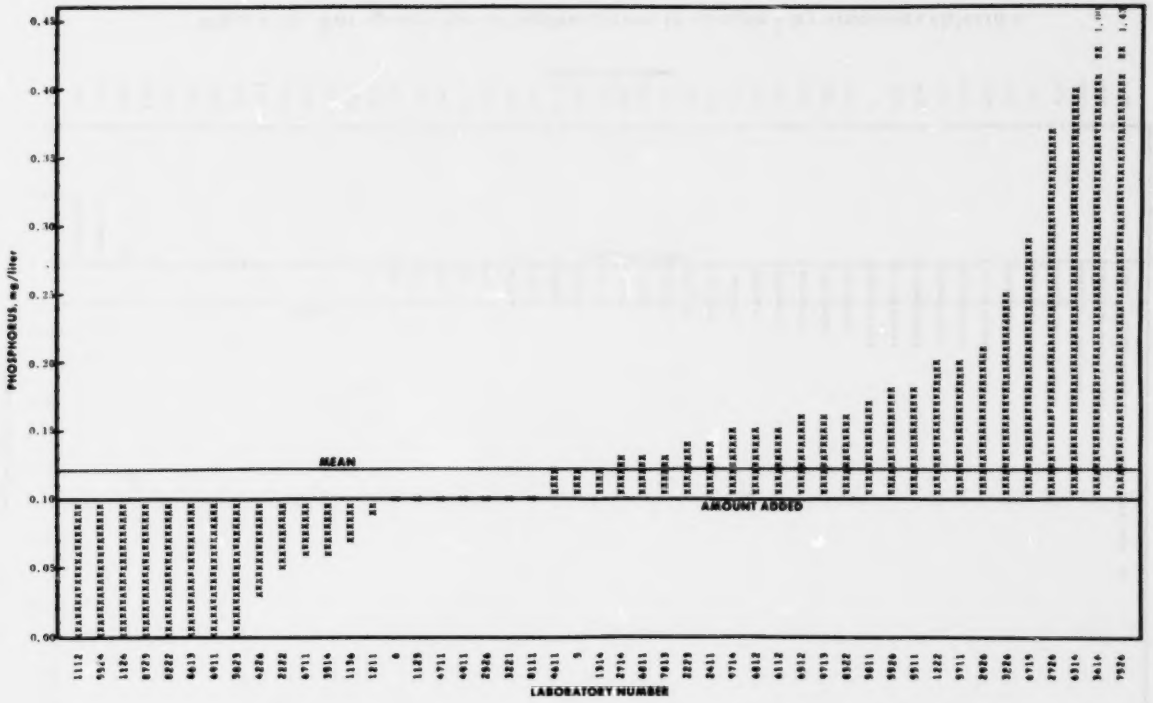

Figure 17. Bar graph for orthophosphate in sample 1 by vanadomolybdate.

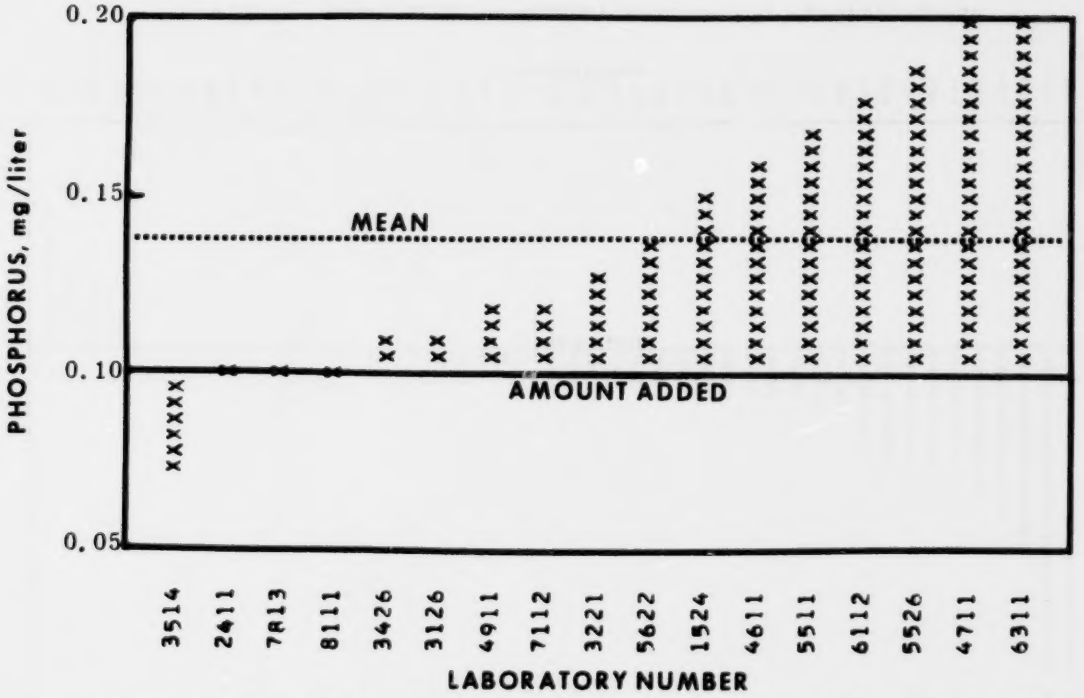

Figure 18. Bar graph for orthophosphate in sample 1 by ammonium molybdate with bismuth.

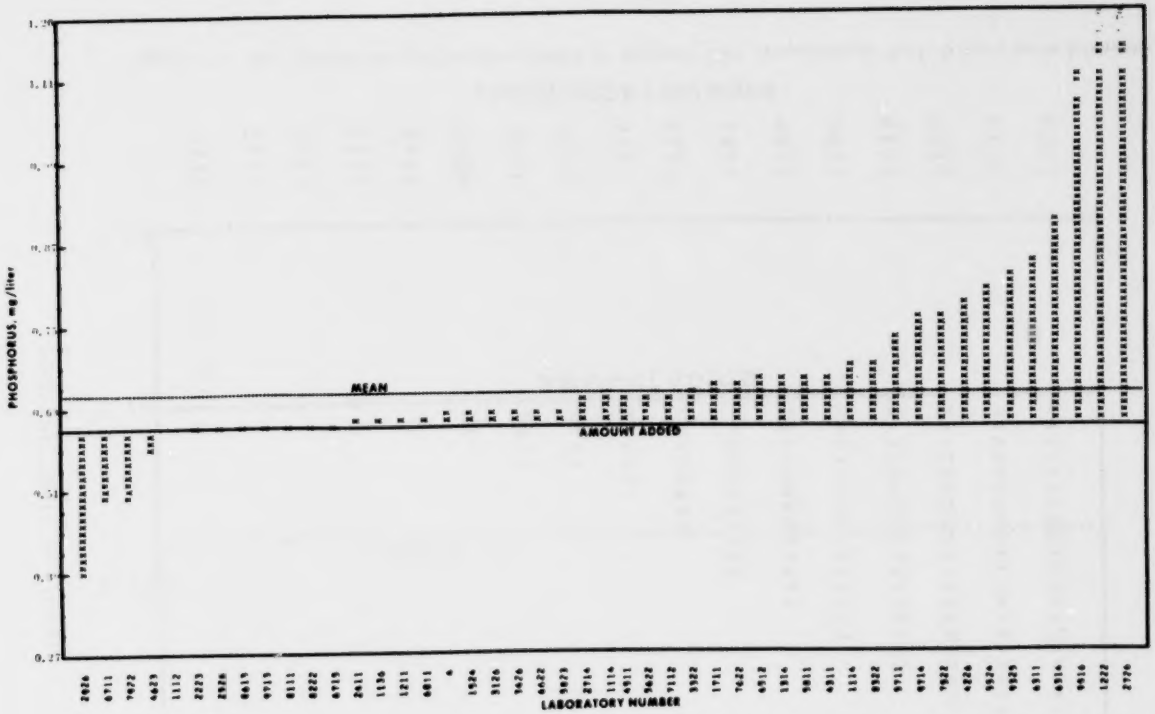

Figure 19. Bar graph for orthophosphate in sample 2 by stannous chloride.

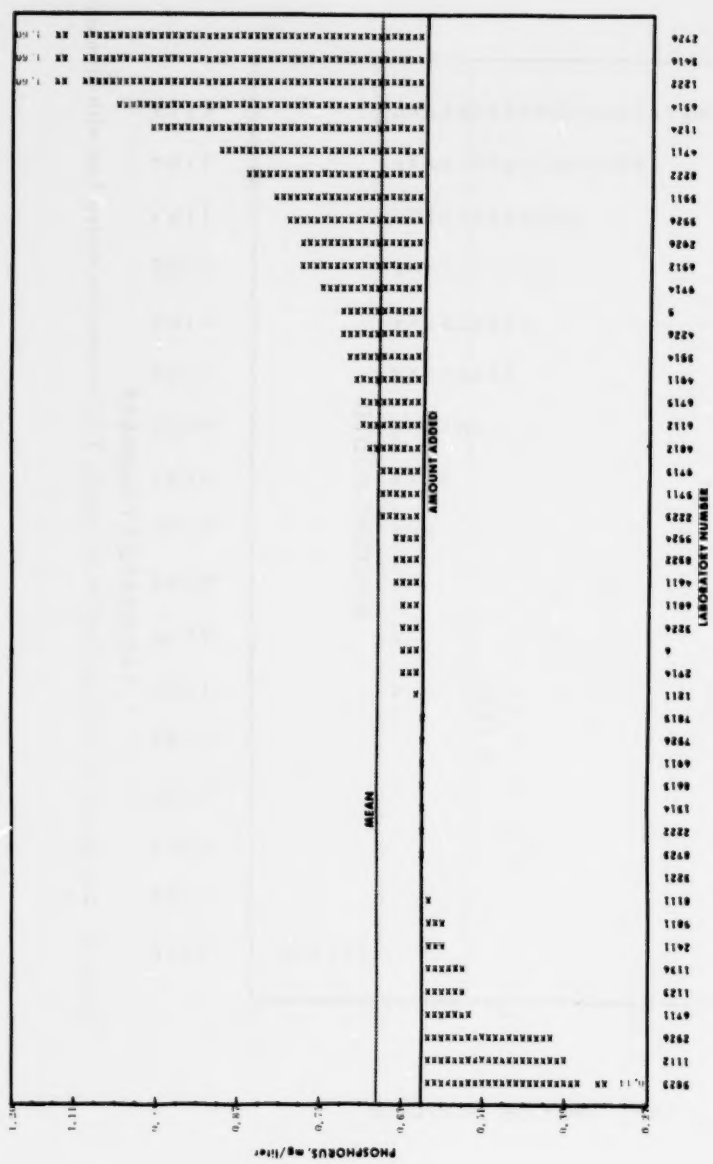

Figure 20. Bar graph for orthophosphate in sample 2 by vanadomolybdate.

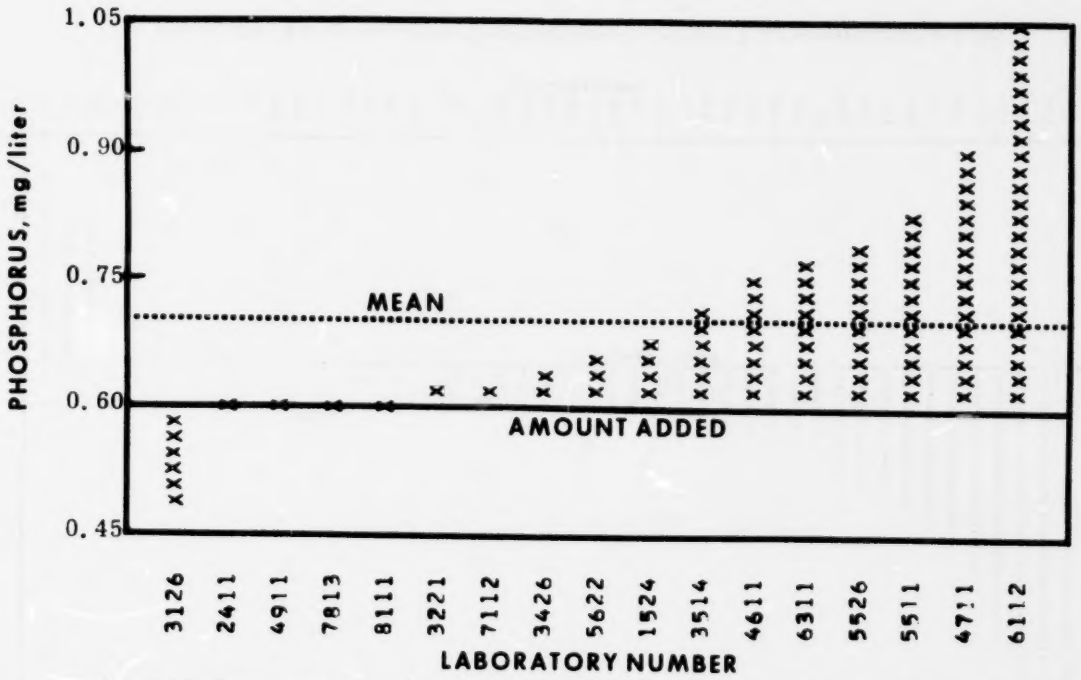

Figure 21. Bar graph for orthophosphate in sample 2 by ammonium molybdate with bismuth.

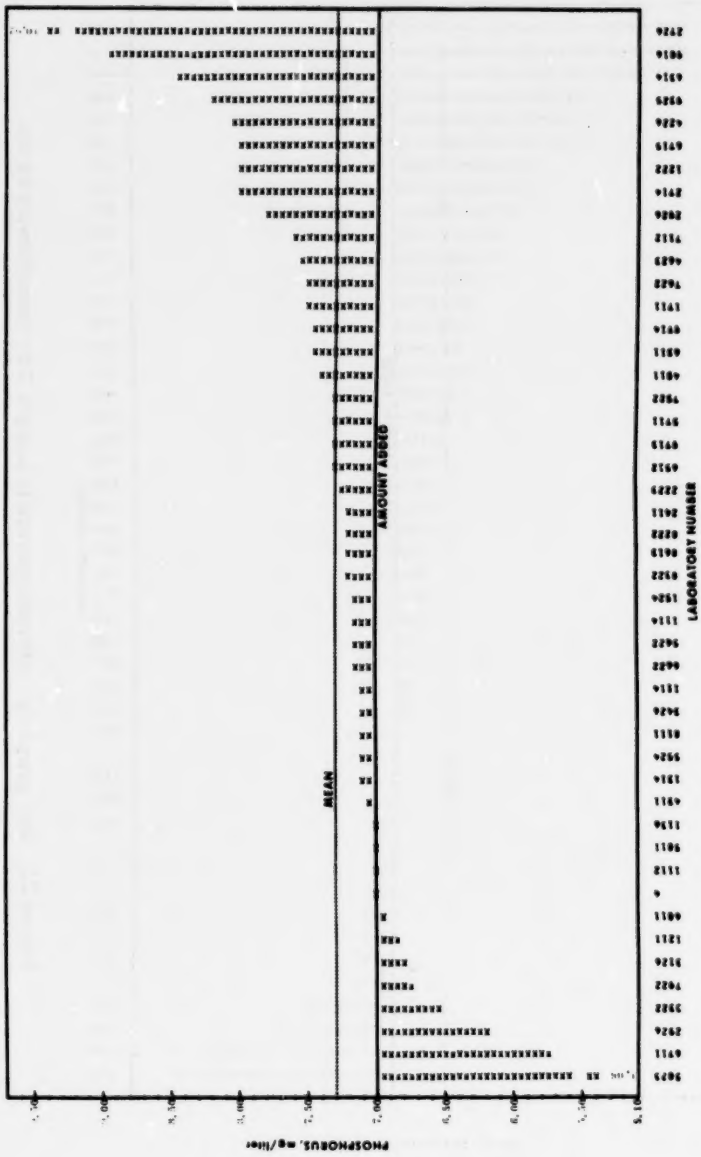

Figure 22. Bar graph for orthophosphate in sample 3 by stannous chloride.

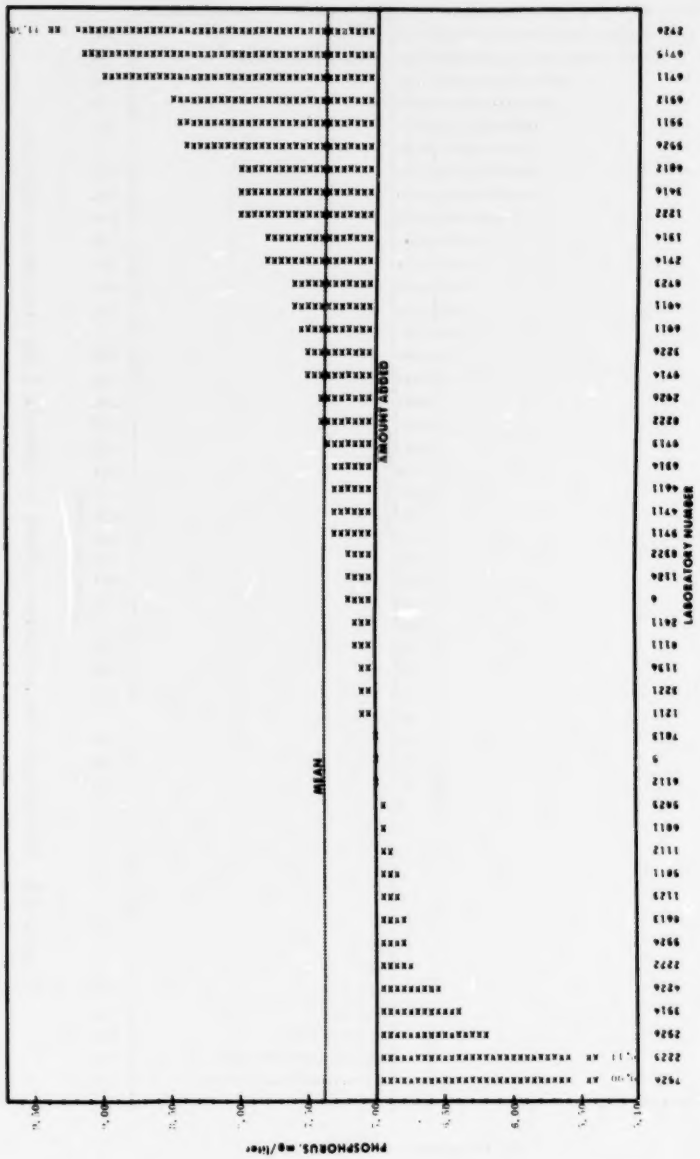

Figure 23. Bar graph for orthophosphate in sample 3 by vanadomolybdate.

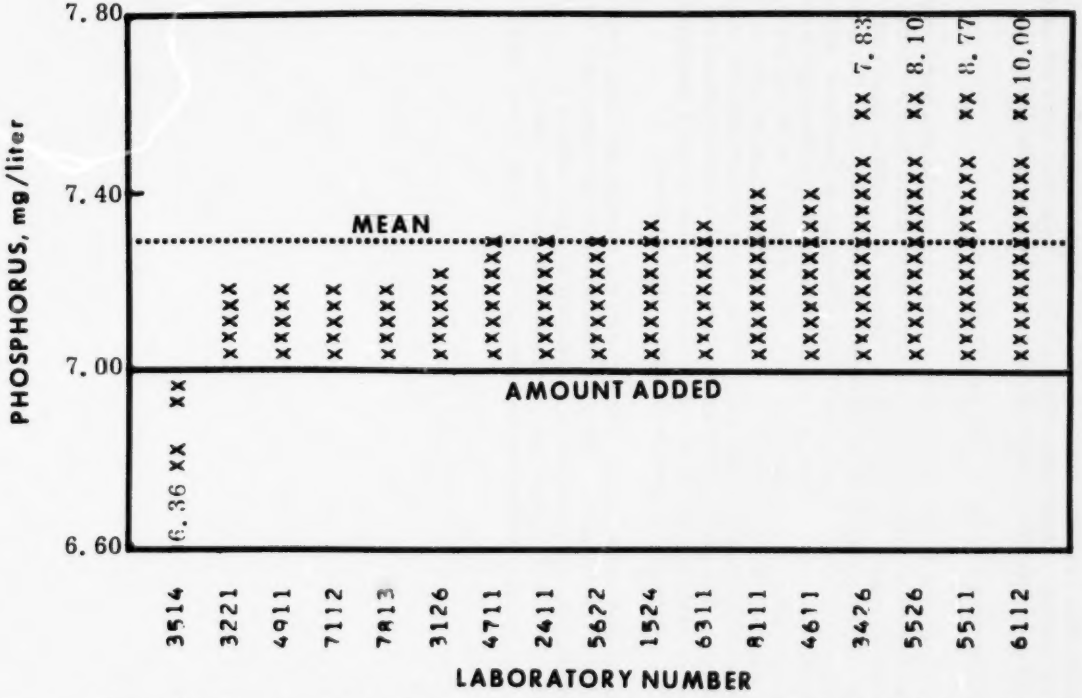

Figure 24. Bar graph for orthophosphate in sample 3 by ammonium molybdate with bismuth.

and precision. Four analysts used the Murphy and Riley single-solution reagent and four used the AutoAnalyzer with stannous chloride, with mediocre results. These latter three methods, however, were not used by enough participants to permit any firm conclusions to be drawn.

POLYPHOSPHATE (Table 5, Figures 25-33)

No significant difference in accuracy for any sample was found among the three requested methods, but, as might be expected from the orthophosphate discussion, the tendency was toward low results, apparently owing to slight hydrolysis of polyphosphate to orthophosphate. For sample 3 (3.00 mg/liter) there was no significant difference in precision, although the bismuth-catalyzed ammonium molybdate method appears slightly more accurate and precise than the other two. For sample 1 (0.08 mg/liter), and for sample 2 (0.30 mg/liter), however, both the stannous chloride and bismuth-catalyzed methods are significantly more precise than the vanadomolybdate. The previous Water Nutrients No. 1 study also found the stannous chloride method to be accurate and precise at small concentrations, but somewhat less precise at large concentrations.

The poorest results were obtained with the Murphy and Riley reagent, used by three participants. The AutoAnalyzer used with stannous chloride by three participants produced good accuracy and precision at 3.00 mg/liter (sample 3) but became less accurate at the smaller concentrations. Again, no firm conclusions can be drawn from only these three values.

Table 5. SUMMARY OF POLYPHOSPHATE RESULTS

Method	Number of results	Number of outliers	Mean	Mean error	Std. Dev.	Rel. error	Rel. std. dev.	95% tol. limits
	Sample 1, 0.08 mg/liter phosphorus							
Hydrolysis and stannous chloride[a]	39	2	0.0700	-0.010	0.0421	12.5	60.1	±0.10
Hydrolysis and vanadomolybdate[a]	37	1	0.0859	+0.006	0.0918	7.4	106.8	±0.23
Ammonium molybdate and bismuth[a]	18	0	0.0850	+0.005	0.0588	6.2	69.2	±0.17
Hydrolysis and Murphy & Riley reagent	3	0	0.0200	-0.060	0.0200	75.0	100.0	±0.20
AutoAnalyzer with stannous chloride	3	0	0.0533	-0.027	0.0153	33.3	28.6	±0.15
	Sample 2, 0.30 mg/liter phosphorus							
Hydrolysis and stannous chloride[a]	36	5	0.2350	-0.065	0.1119	21.7	47.6	±0.28
Hydrolysis and vanadomolybdate[a]	38	1	0.2582	-0.042	0.1717	14.0	66.5	±0.42
Ammonium molybdate and bismuth[a]	15	3	0.2220	-0.078	0.0959	26.0	43.2	±0.28
Hydrolysis and Murphy & Riley reagent	3	0	0.1333	-0.167	0.0473	55.6	35.4	±0.47
AutoAnalyzer with stannous chloride	3	0	0.2233	-0.077	0.1069	25.6	47.9	±1.06
	Sample 3, 3.00 mg/liter phosphorus							
Hydrolysis and stannous chloride[a]	38	3	2.3158	-0.684	0.8665	22.8	37.4	±2.13
Hydrolysis and vanadomolybdate[a]	37	2	2.2957	-0.704	0.8291	23.5	36.1	±2.05
Ammonium molybdate and bismuth[a]	17	1	2.6000	-0.400	0.6629	13.3	25.5	±1.89
Hydrolysis and Murphy & Riley reagent	3	0	1.6367	-1.363	0.5221	45.4	31.9	±5.18
AutoAnalyzer with stannous chloride	3	0	2.6467	-0.353	0.3009	11.8	11.4	±2.98

[a]Methods requested by ARS.

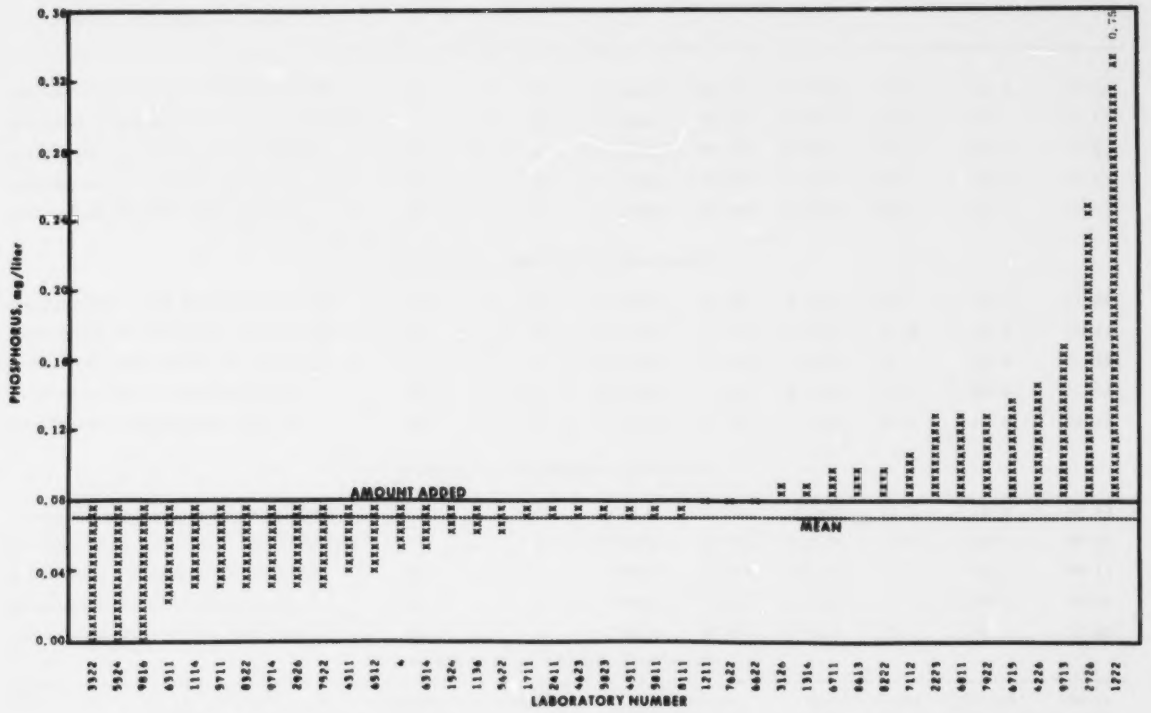

Figure 25. Bar graph for polyphosphate in sample 1 by stannous chloride after hydrolysis.

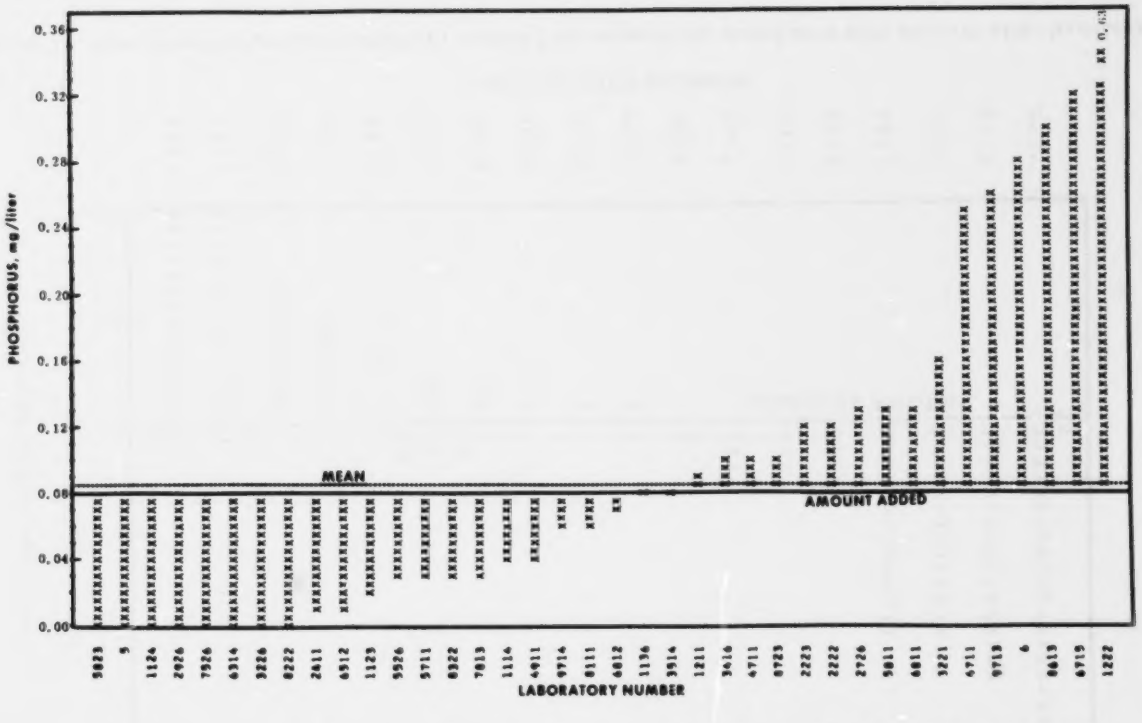

Figure 26. Bar graph for polyphosphate in sample 1 by vanadomolybdate after hydrolysis.

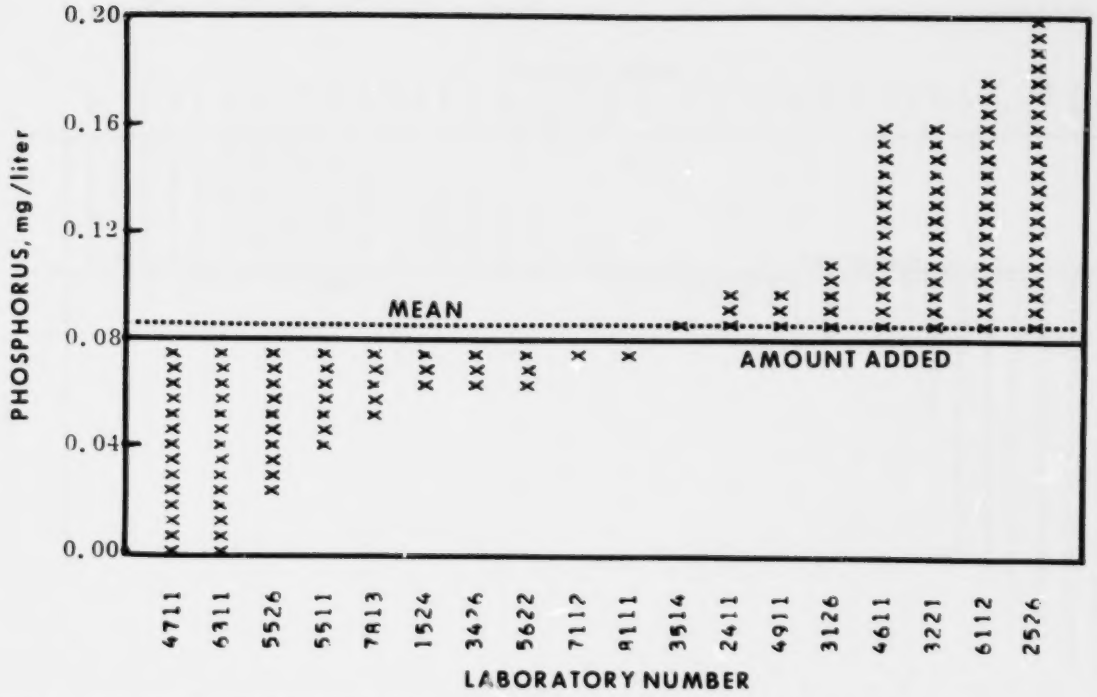

Figure 27. Bar graph for polyphosphate in sample 1 by ammonium molybdate with bismuth after hydrolysis.

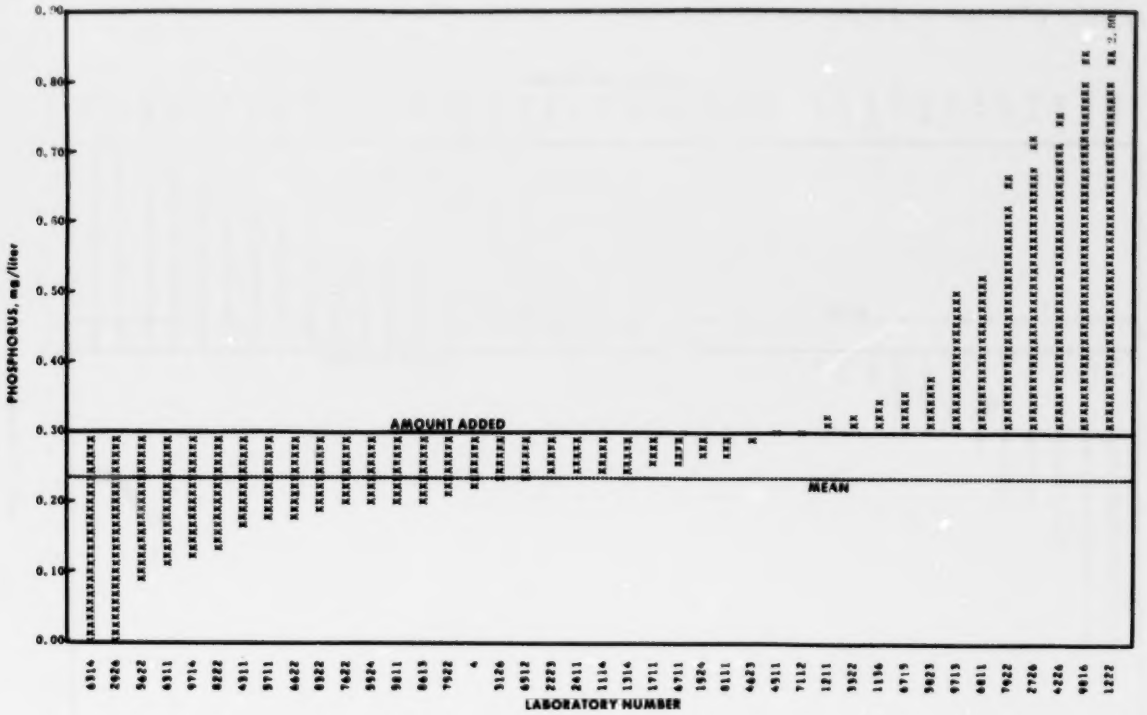

Figure 28. Bar graph for polyphosphate in sample 2 by stannous chloride after hydrolysis.

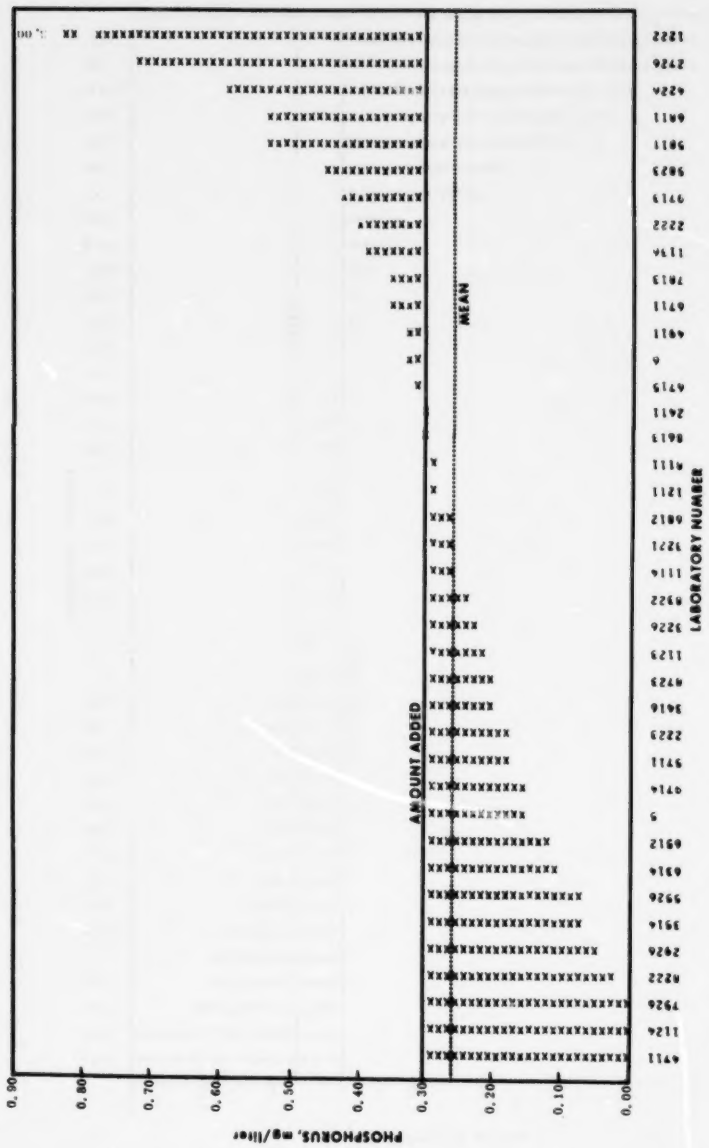

Figure 29. Bar graph for polyphosphate in sample 2 by vanadomolybdate after hydrolysis.

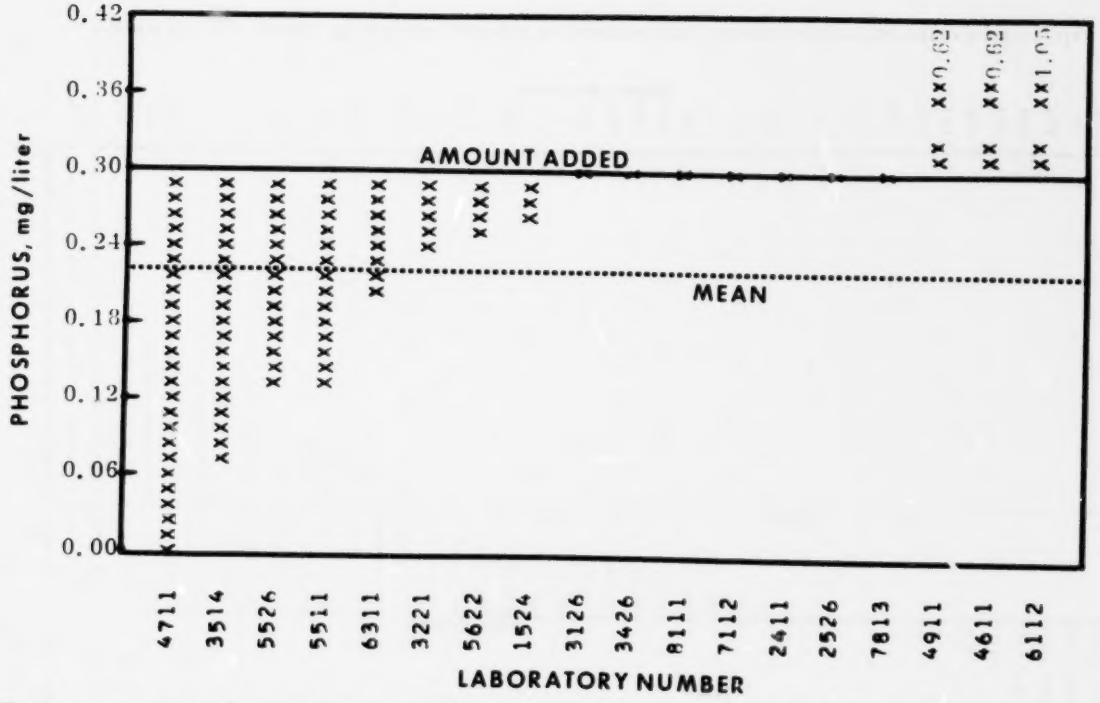

Figure 30. Bar graph for polyphosphate in sample 2 by ammonium molybdate with bismuth after hydrolysis.

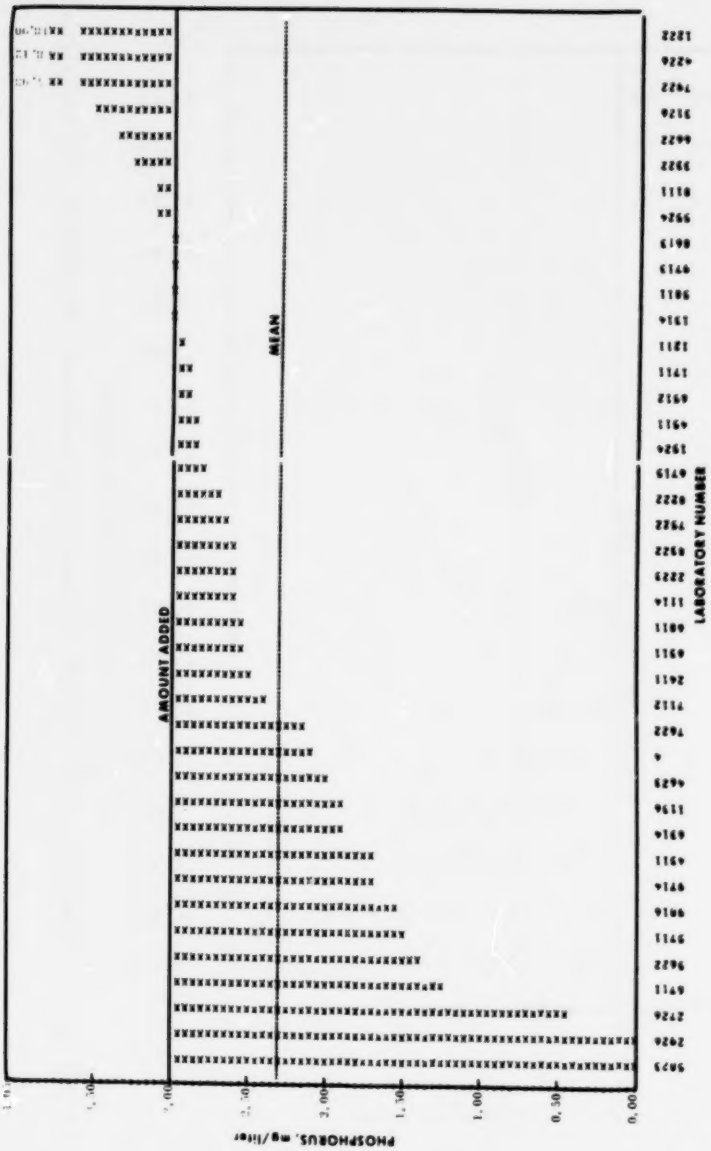

Figure 31. Bar graph for polyphosphate in sample 3 by stannous chloride after hydrolysis.

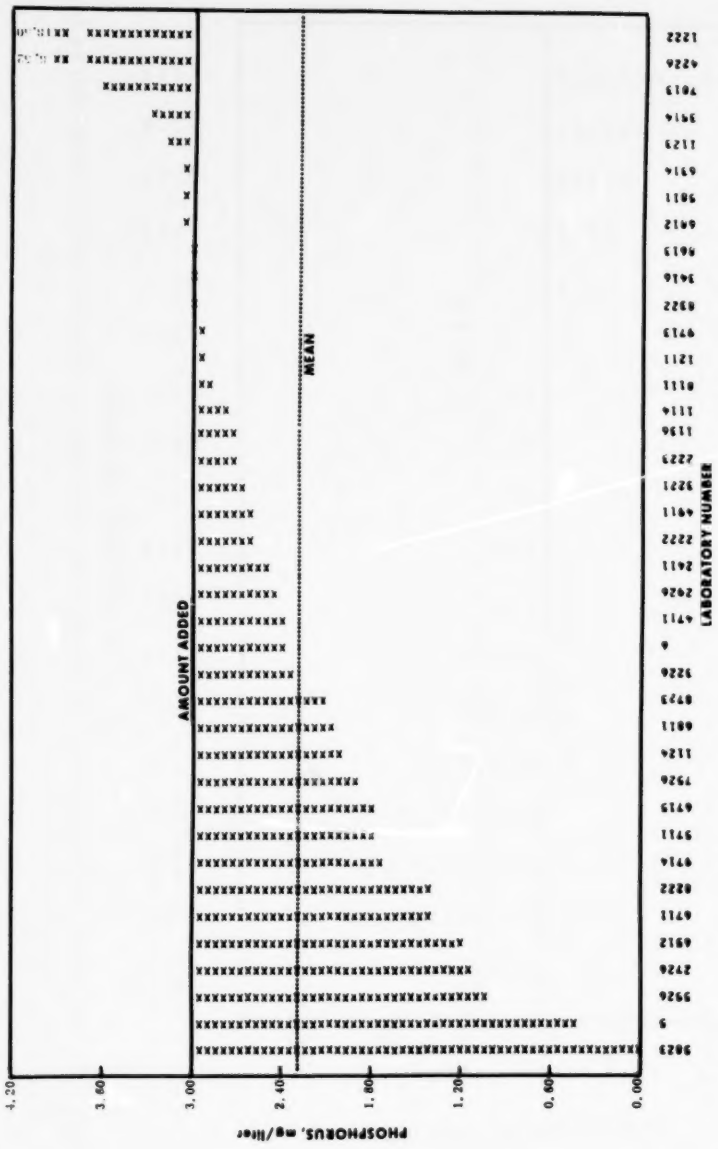

Figure 32. Bar graph for polyphosphate in sample 3 by vanadomolybdate after hydrolysis.

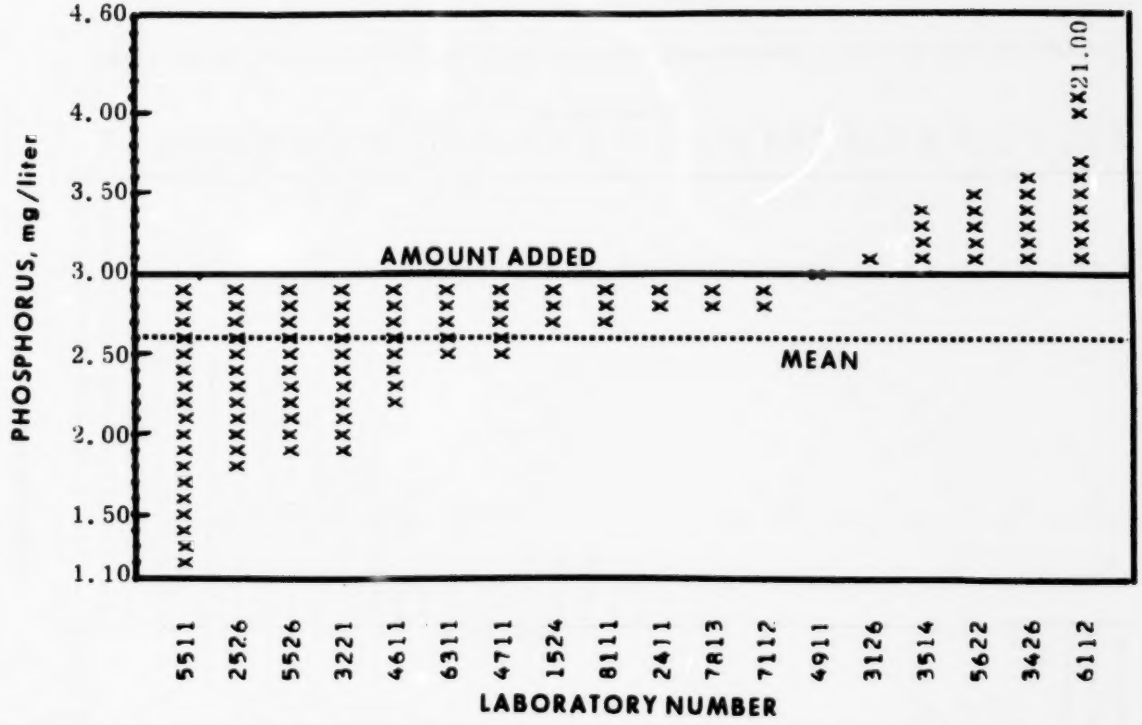

Figure 33. Bar graph for polyphosphate in sample 3 by ammonium molybdate with bismuth after hydrolysis.

TOTAL PHOSPHATE (Table 6, Figures 34-45)

In sample 1 (0.21 mg/liter) and sample 2 (0.99 mg/liter), the sulfuric-nitric digestion with stannous chloride was significantly more precise than the same digestion used with vanadomolybdate, but there was no difference in accuracy. In these two samples, the persulfate digestion with stannous chloride was also more precise than the same digestion used with vanadomolybdate, but for sample 2 (0.99 mg/liter) the persulfate digestion with vanadomolybdate was more accurate. In both samples, the persulfate digestion with vanadomolybdate was more precise than the sulfuric-nitric digestion with vanadomolybdate. In other words, with the vanadomolybdate colorimetric procedure, the persulfate digestion is superior to the sulfuric-nitric. With the stannous chloride colorimetric procedure, there was no significant difference in precision between the sulfuric-nitric and persulfate digestions in samples 1 and 2. In sample 3 (10.23 mg/liter), however, the sulfuric-nitric digestion was more precise but there was no difference in accuracy. For all three samples, the greatest precision was shown by the Murphy and Riley procedure preceded by persulfate digestion. However, the difference was significant only for sample 1, and since only four values were obtained by this technique, the reliability of the data is questionable. The vanadomolybdate procedure preceded by perchloric acid digestion was used by six participants, and the precision and accuracy obtained were fair for samples 2 and 3 but poor for sample 1, even though two of the six values were rejected as outliers. As before, the amount of data available on this method is too sparse to provide a reliable evaluation.

The method of choice appears to be the sulfuric-nitric digestion procedure followed by the stannous chloride colorimetric determination.

Table 6. SUMMARY OF TOTAL PHOSPHATE RESULTS

Method	Number of results	Number of outliers	Mean	Mean error	Std. dev.	Rel. error	Rel. std. dev.	95% tol. limits
Sample 1, 0.21 mg/liter phosphorus								
Perchloric and vanadomolybdate[a]	4	2	0.3050	+0.095	0.1021	45.2	33.5	±0.65
Sulfuric-nitric and stannous chloride[a]	20	2	0.2075	-0.003	0.0431	1.2	20.8	±0.12
Sulfuric-nitric and vanadomolybdate[a]	23	0	0.2539	+0.044	0.1666	20.9	65.6	±0.45
Persulfate and stannous chloride	29	1	0.1907	-0.019	0.0535	9.2	28.1	±0.14
Persulfate and vanadomolybdate	32	2	0.2066	-0.003	0.1152	1.6	55.8	±0.29
Persulfate and Murphy & Riley reagent	4	1	0.2000	-0.010	0.0082	4.8	4.1	±0.05
Sample 2, 0.99 mg/liter phosphorus								
Perchloric and vanadomolybdate[a]	5	1	1.0160	+0.026	0.2061	2.6	20.3	±1.05
Sulfuric-nitric and stannous chloride[a]	17	5	1.0218	+0.032	0.0903	3.2	8.8	±0.26
Sulfuric-nitric and vanadomolybdate[a]	22	1	0.9955	+0.005	0.4709	0.6	47.3	±1.27
Persulfate and stannous chloride	30	0	0.8687	-0.121	0.1296	12.3	14.9	±0.33
Persulfate and vanadomolybdate	32	2	0.9669	-0.023	0.2307	2.3	23.9	±0.58
Persulfate and Murphy & Riley reagent	5	1	0.9800	-0.010	0.0765	1.0	7.8	±0.39
Sample 3, 10.23 mg/liter phosphorus								
Perchloric and vanadomolybdate[a]	6	0	10.0067	-0.223	1.1678	2.2	11.7	±5.15
Sulfuric-nitric and stannous chloride[a]	19	3	10.2695	+0.039	0.7716	0.4	7.5	±2.15
Sulfuric-nitric and vanadomolybdate[a]	20	3	10.1925	-0.038	0.7127	0.4	7.0	±1.96
Persulfate and stannous chloride	29	1	9.7941	-0.436	1.1286	4.3	11.5	±2.89
Persulfate and vanadomolybdate	31	3	10.1981	-0.032	0.6595	0.3	6.5	±1.67
Persulfate and Murphy & Riley reagent	5	1	10.3940	+0.164	0.3565	1.6	3.4	±1.81

[a]Methods requested by ARS

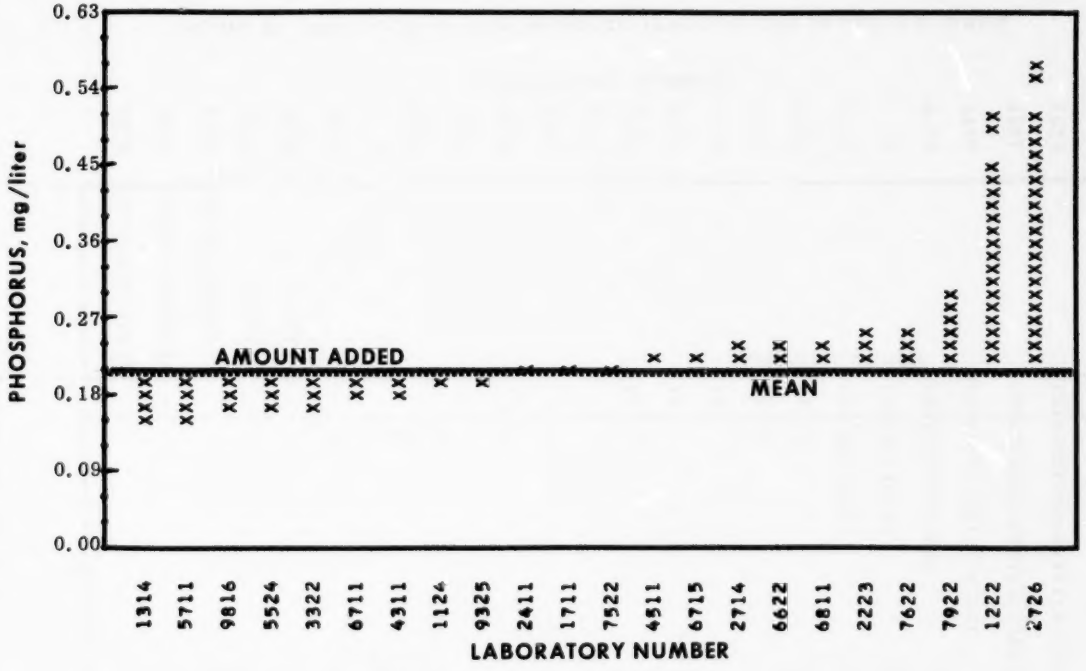

Figure 34. Bar graph for total phosphate in sample 1 by stannous chloride after sulfuric-nitric acid digestion.

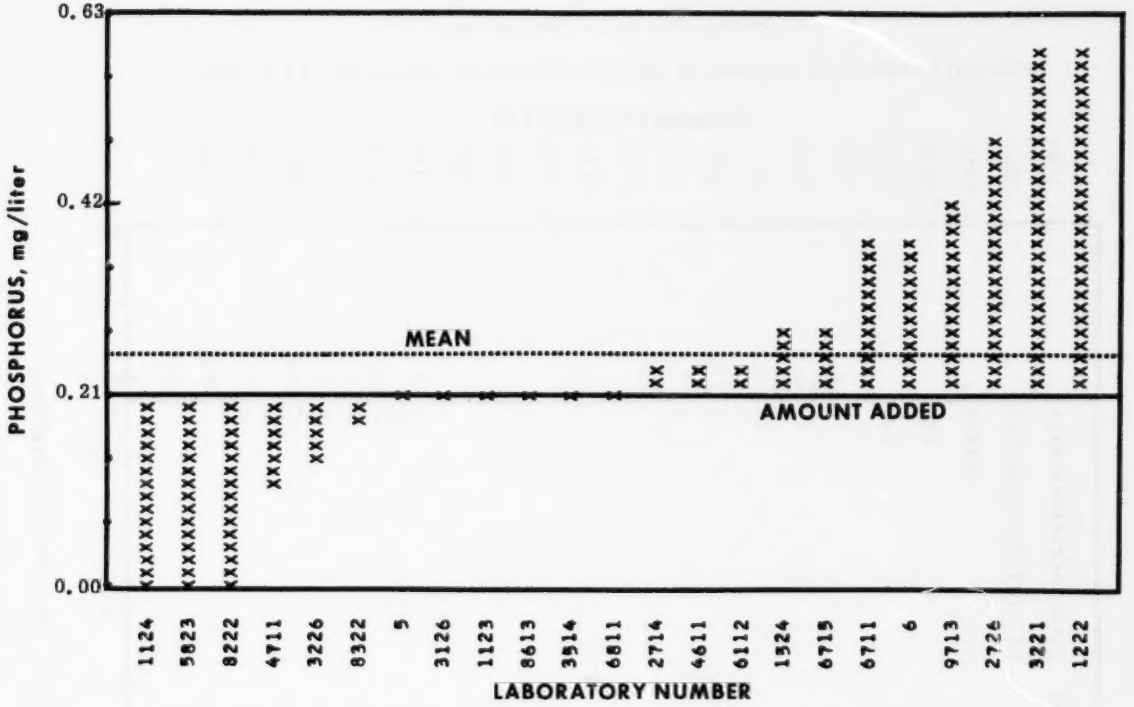

Figure 35. Bar graph for total phosphate in sample 1 by vanadomolybdate after sulfuric-nitric acid digestion.

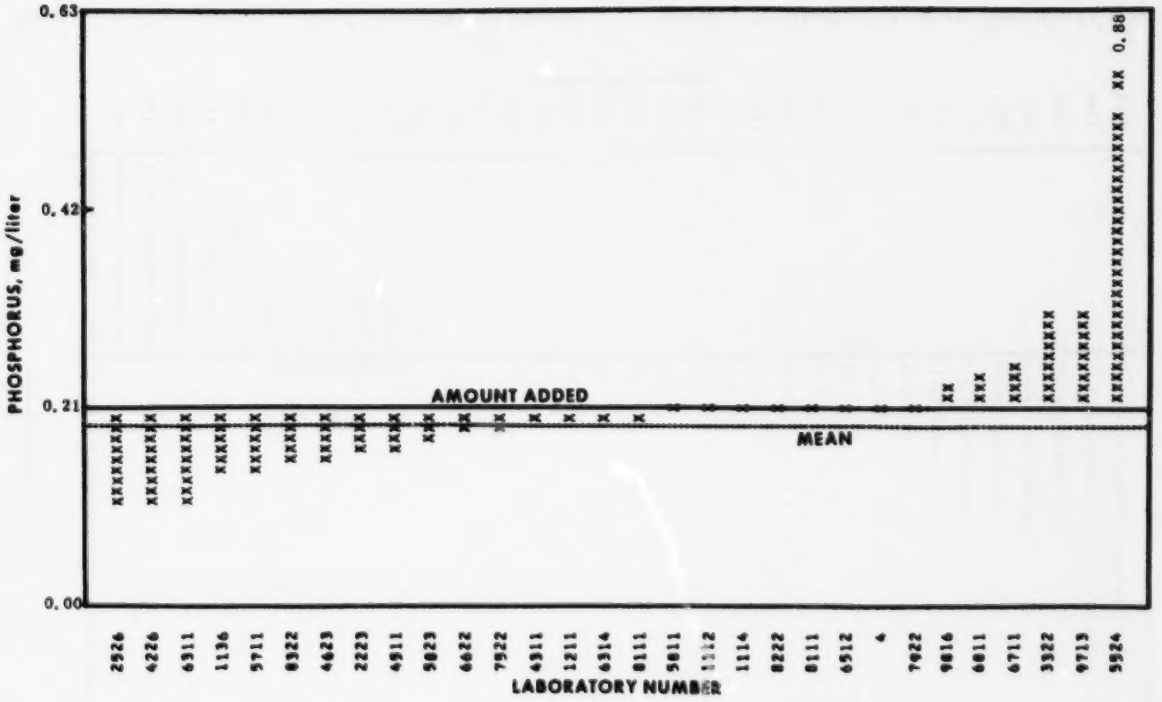

Figure 36. Bar graph for total phosphate in sample 1 by stannous chloride after persulfate digestion.

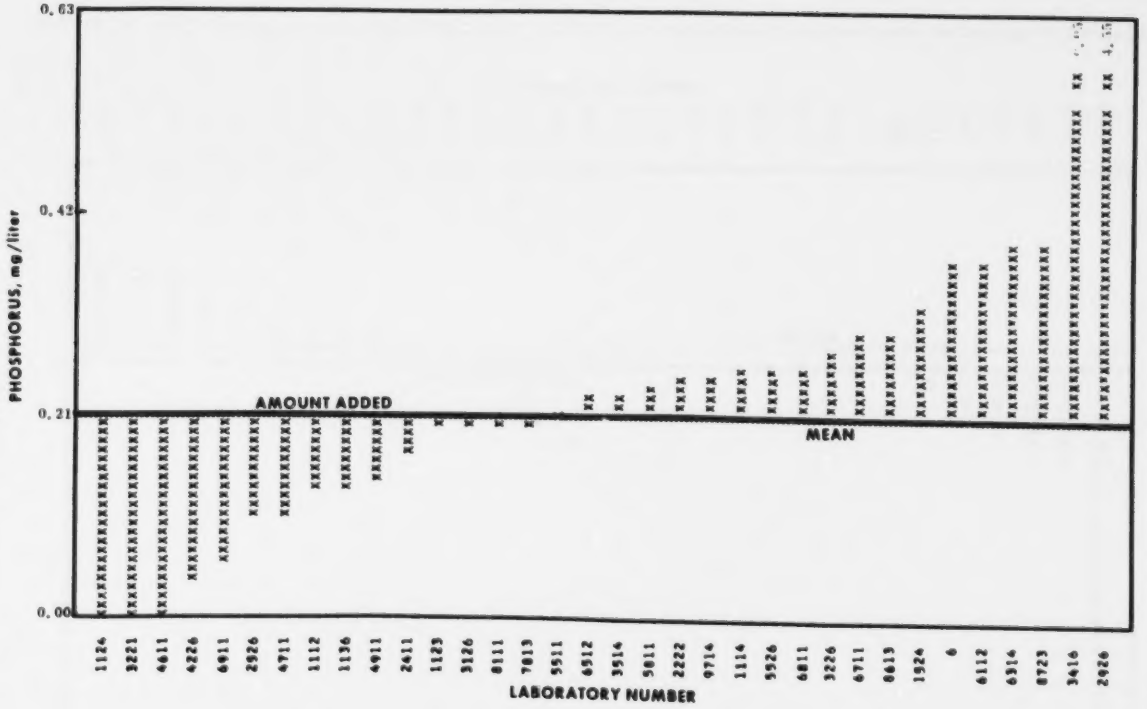

Figure 37. Bar graph for total phosphate in sample 1 by vanadomolybdate after persulfate digestion.

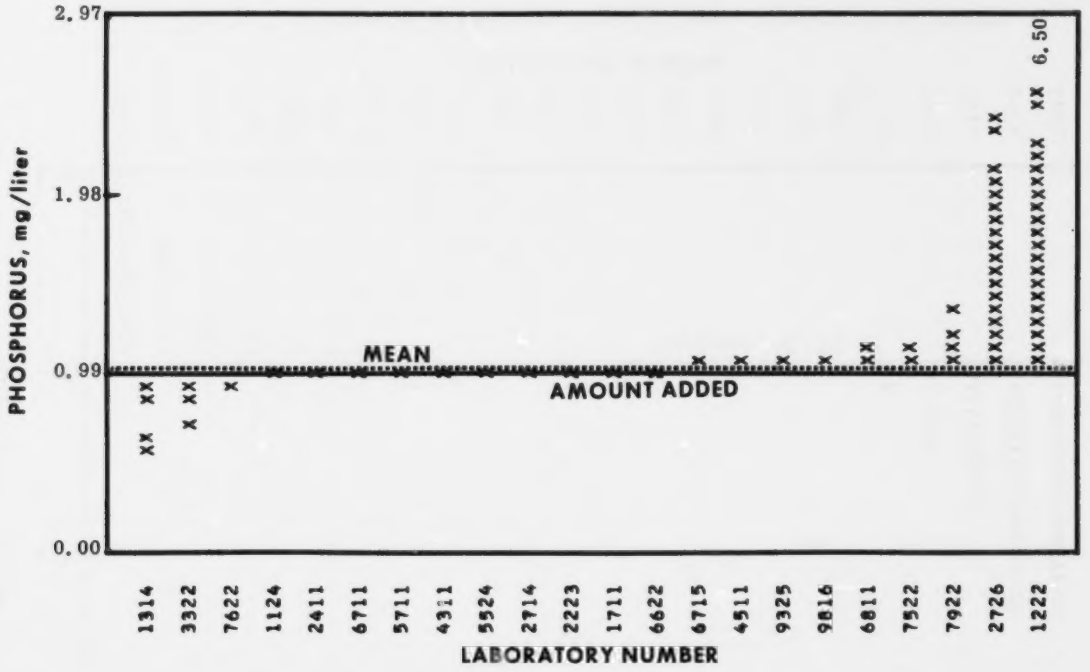

Figure 38. Bar graph for total phosphate in sample 2 by stannous chloride after sulfuric-nitric acid digestion.

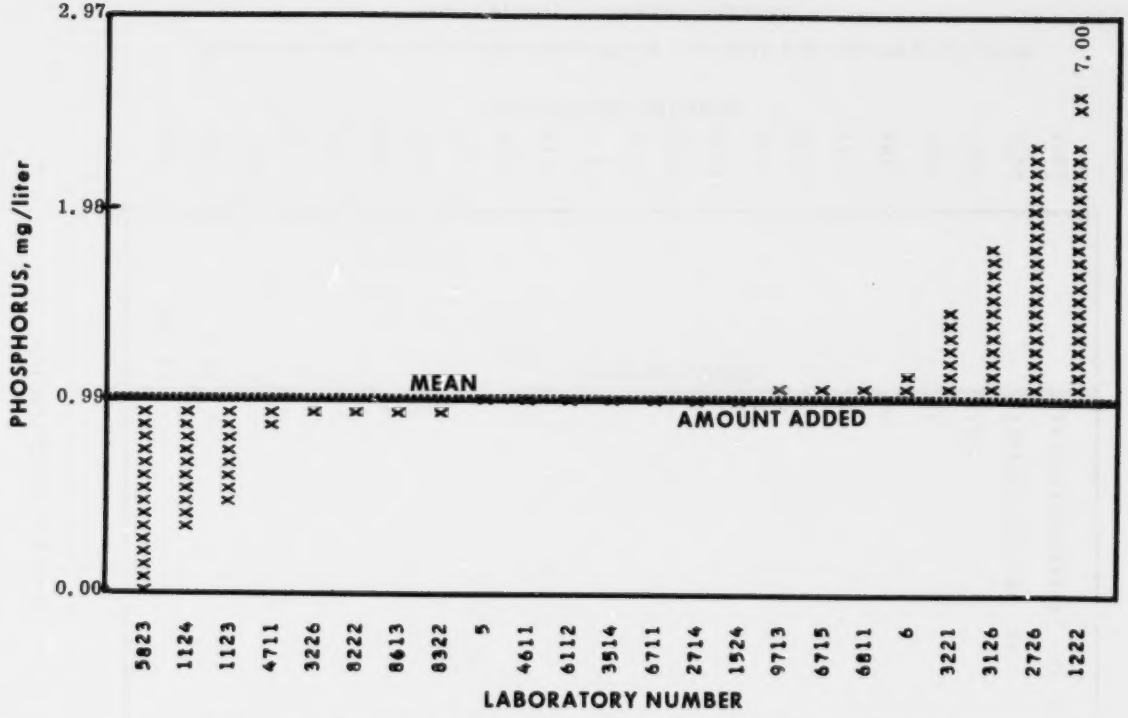

Figure 39. Bar graph for total phosphate in sample 2 by vanadomolybdate after sulfuric-nitric acid digestion.

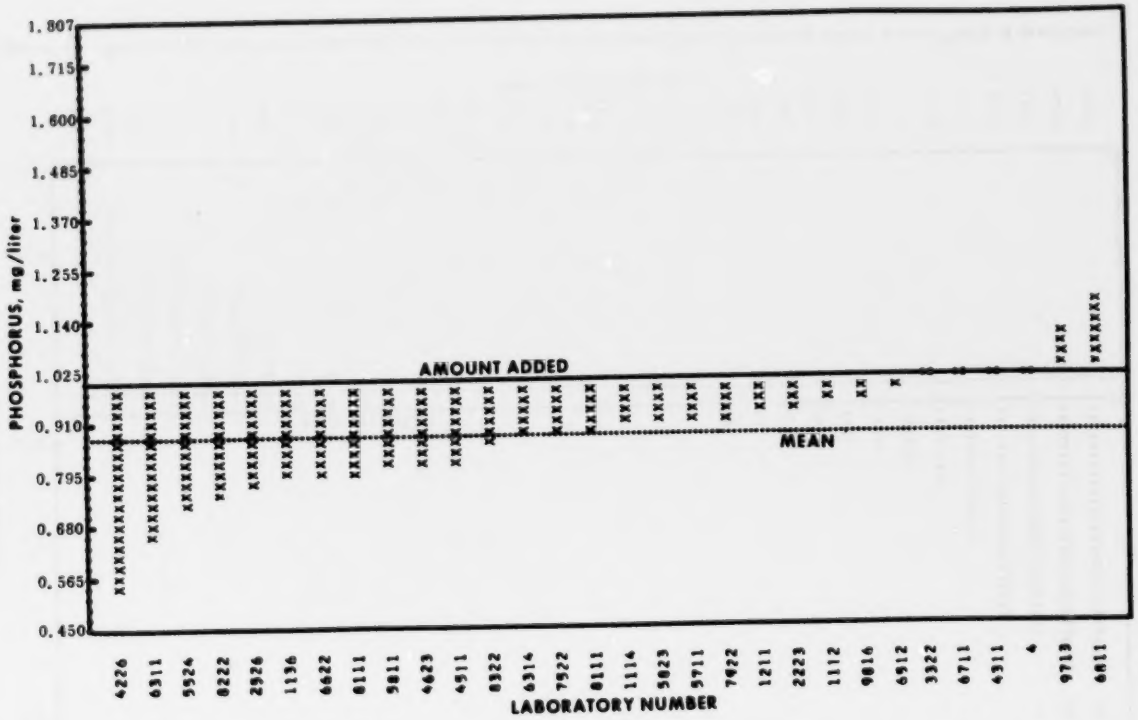

Figure 40. Bar graph for total phosphate in sample 2 by stannous chloride after persulfate digestion

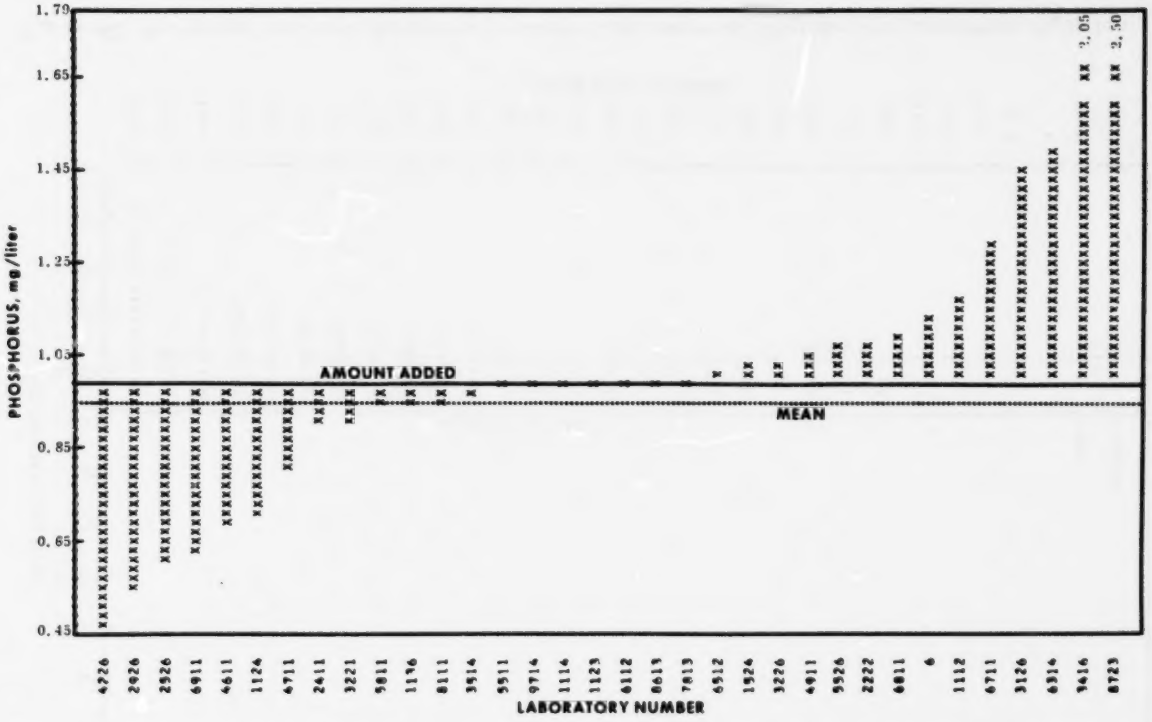

Figure 41. Bar graph for total phosphate in sample 2 by vanadomolybdate after persulfate digestion.

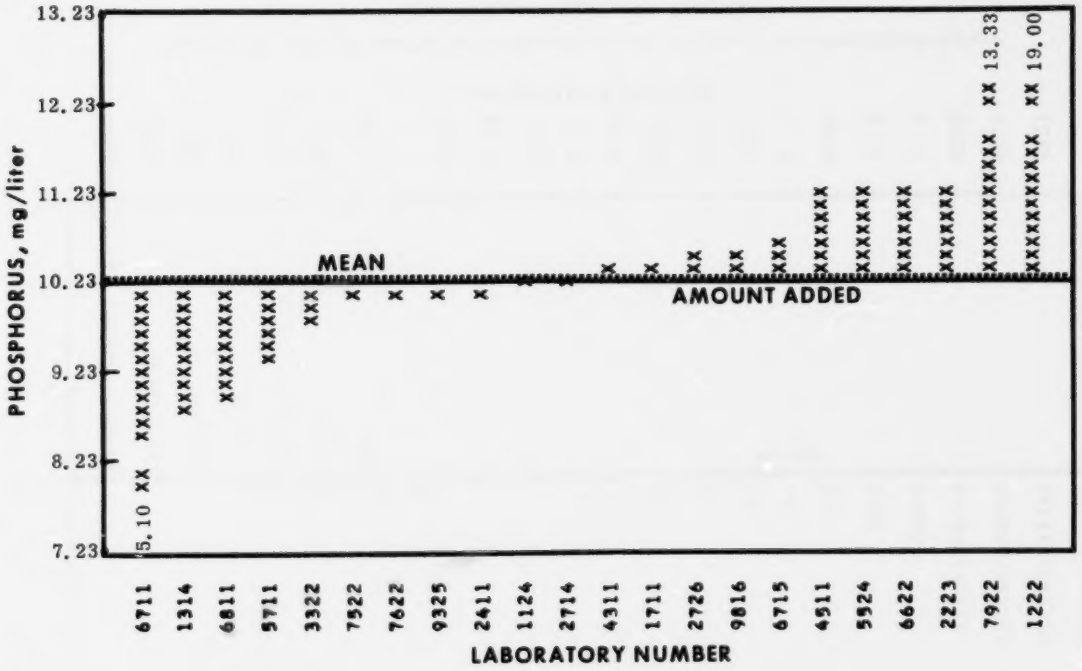

Figure 42. Bar graph for total phosphate in sample 3 by stannous chloride after sulfuric-nitric acid digestion.

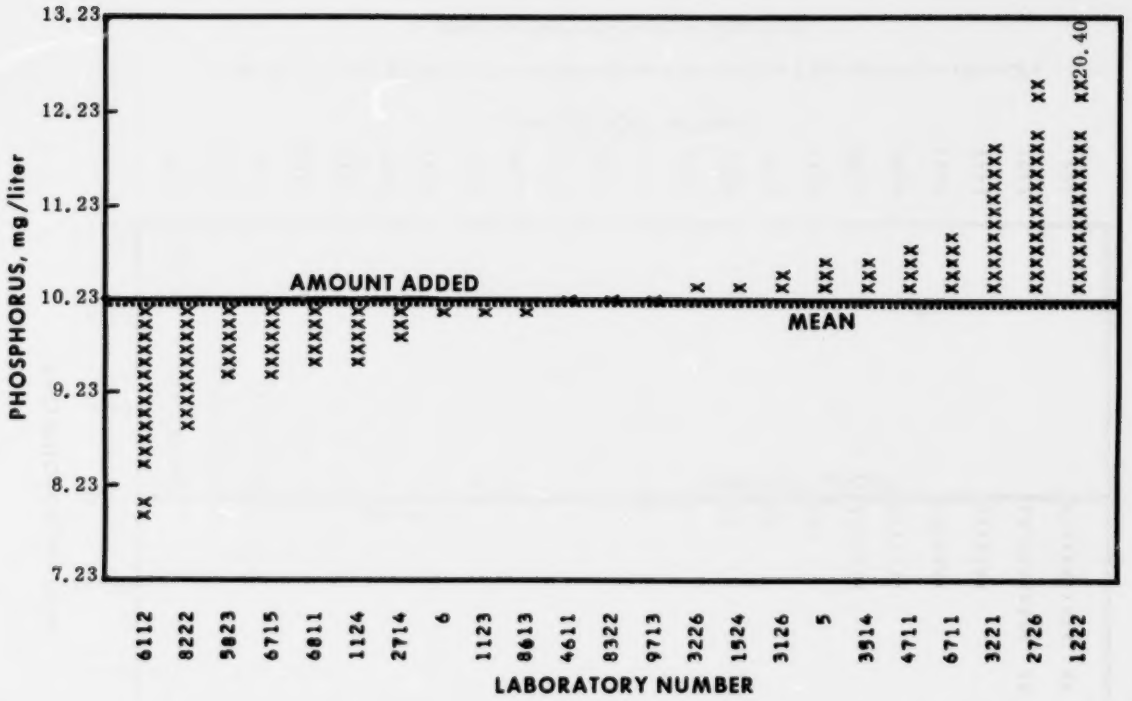

Figure 43. Bar graph for total phosphate in sample 3 by vanadomolybdate after sulfuric-nitric acid digestion.

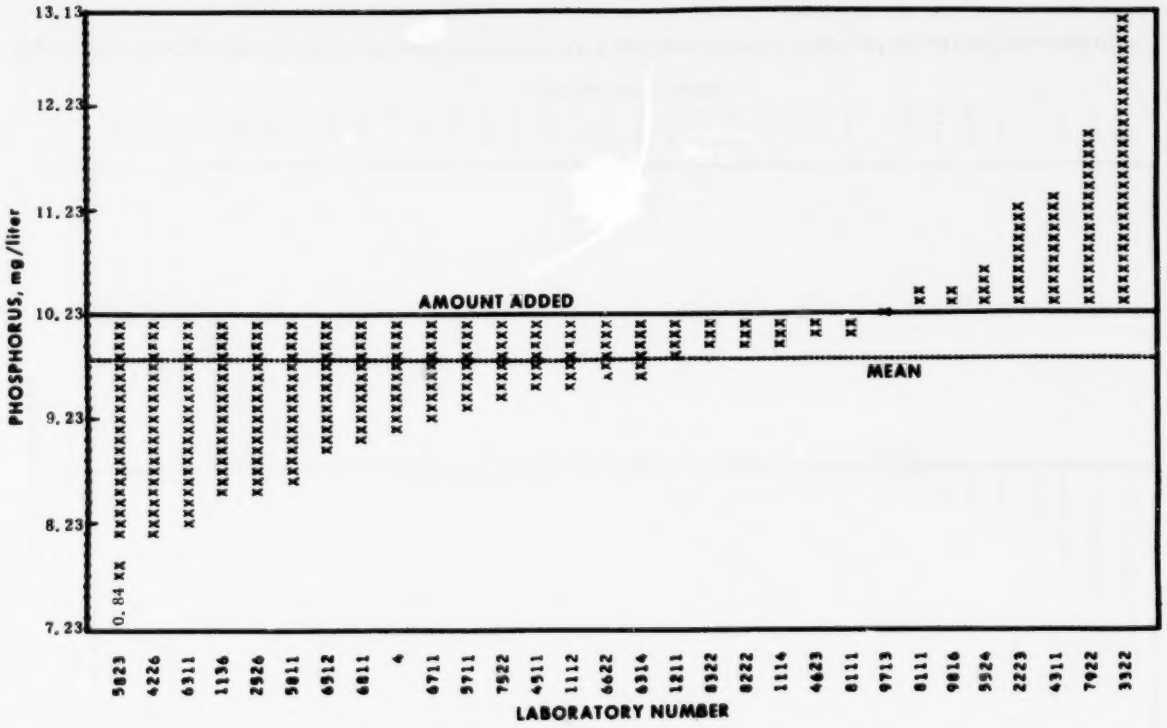

Figure 44. Bar graph for total phosphate in sample 3 by stannous chloride after persulfate digestion.

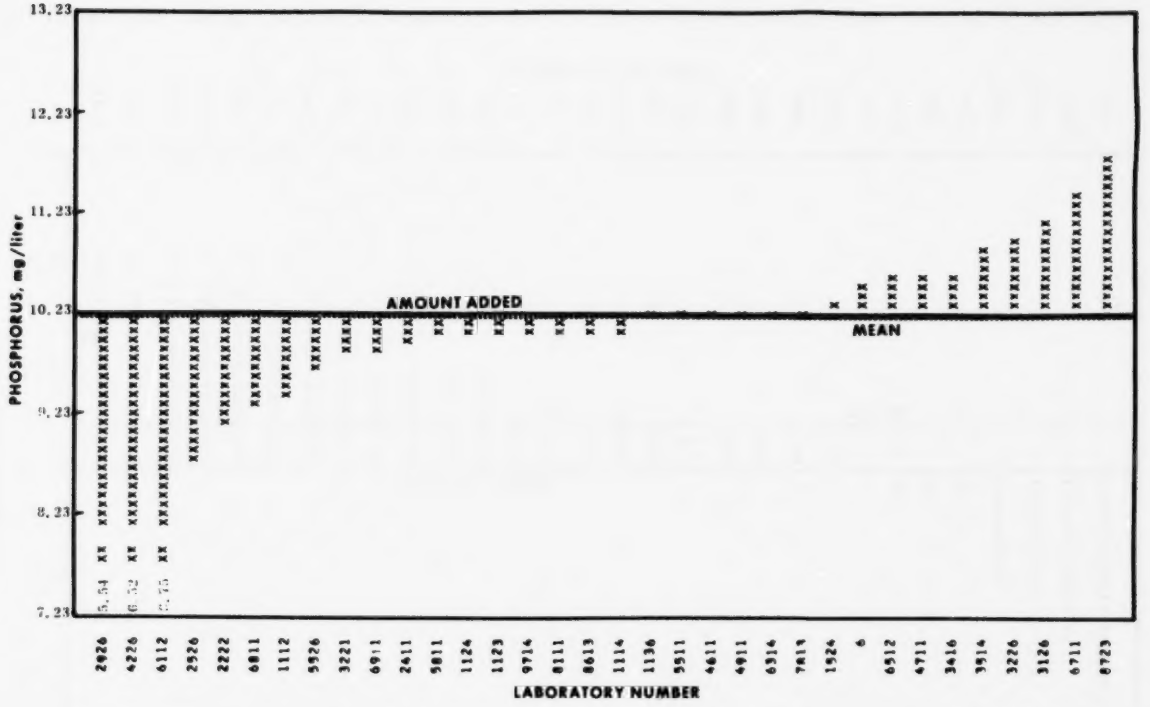

Figure 45. Bar graph for total phosphate in sample 3 by vanadomolybdate after persulfate digestion.

COMMENTS OF THE PARTICIPANTS

AMMONIA NITROGEN

Eight participants commented that the results obtained with the phenate method were erratic owing to inability to reproduce the standard curve. One participant remarked that they had been using the phenate method for about a year and had found that new reagent must be prepared each working day. They also found that the rate of addition of the phenate reagent was critical. Another commented that Beer's law does not hold above about 0.5 mg per liter. Two participants commented that ammonia contamination was apparently a problem since it appeared that mixing of the solutions in an open beaker resulted in absorption of ammonia from the atmosphere. Another commented that when distillation is used, the boric acid collecting medium interfered with the color development, and weak hydrochloric acid was therefore substituted.

NITRATE NITROGEN

Four participants commented that they had difficulty purifying the chromotropic acid. Two stated that they could not obtain a pure acid and another stated that it was necessary to reduce the volume to 50 ml before crystallization took place. Two others commented that they had difficulty with the antimony reagent since dissolution took several hours and the reagent tended to precipitate on standing.

Two participants commented that the milling of the cadmium and preparation of the column were very tedious and time consuming, but the sensitivity of the method appeared to make it worth the trouble.

The modified brucine method was also found to be time consuming and erratic by two of the participants.

One participant remarked that the hydrazine reduction method using an AutoAnalyzer was preferred because of the large number of samples which could be analyzed.

ORTHOPHOSPHATE

Two participants commented and two others called ARS by telephone in regard to the preparation of the vanadate-molybdate reagent. If solution B is prepared by dissolving 1.25 g of ammonium metavanadate in 300 ml of boiled distilled water and then adding 330 ml of concentrated hydrochloric acid and 400 ml of solution A, the mixture cannot be made up to 1 liter, for the volume is obviously already greater. As ARS found, however, if the 1.25 g of ammonium metavanadate is added to 300 ml of distilled water, which is then heated to boiling, some loss occurs owing

to evaporation on heating, and the mixture can then be made up to a final volume of 1 liter. An even better procedure would be to make solution A up in 300 ml rather than 400 ml. Moreover, the statement that the final acid concentration is 0.5N refers to the concentration of the acid in the final mixture of sample and reagents and not to this reagent. One participant stated that he did not get the sensitivity claimed for this reagent.

One participant had difficulty with the stannous chloride method and abandoned it. He stated that the stannous chloride reagent gave too intense a color to measure even when corrected for a blank.

Another participant had difficulty with the bismuth-catalyzed method. He found that the results were erratic when fresh reagents were used but became reproducible after the reagents had aged for several days.

POLYPHOSPHATE

The only comment on the polyphosphate methods was that on mild acid hydrolysis and measurement of the orthophosphate content by the vanadomolybdate method, lower results were obtained than had been obtained for orthophosphate alone. He concluded that the vanadomolybdate method was not, therefore, sensitive enough to measure small concentrations accurately. As a matter of fact, the method states that the minimum detectable concentration is 0.2 mg P/liter.

As a result of several telephone calls and an exchange of letters between ARS and participants in an effort to correct what appeared to be calculation errors, it is evident that widespread confusion exists regarding terminology. For example, on page 77 of the phosphate methods (Appendix C), "Filterable (soluble) acid-hydrolyzable phosphate" is defined as "filtration of whole sample through 0.45 μ membrane filter followed by mild acid hydrolysis of filtrate and orthophosphate determination." Yet on page 85 under "Filterable Condensed Phosphates," the polyphosphate content of the sample is operationally defined as "the difference between the orthophosphate as measured by Method A or B and the phosphate found after mild acid hydrolysis." Perhaps, it would help if the introductory statement would also indicate that acid hydrolyzable phosphate is condensed phosphate and is determined by subtraction of "Filterable (soluble) orthophosphate" from the phosphate obtained after mild acid hydrolysis. Much confusion also resulted because many participants from habit reported their results as PO_4; whereas, the procedures clearly called for P.

TOTAL PHOSPHATE

Two participants commented that it was impossible to neutralize the perchloric acid oxidation solution with (Appendix C, Section 4.4 of "Per-

chloric Acid Digestion") 1N sodium hydroxide. One participant suggested 10N would be more appropriate and the other stated that he used 50 percent sodium hydroxide. Such statements tend to indicate that the analyst did not carry the digestion to the fuming stage; namely, because of the presence of low amounts of organic matter the analyst stopped the digestion short of completion and hence, more sodium hydroxide was required.

One participant noted that the specified 1N sulfuric acid solution was never used in the procedure. Another noted that high blanks were apparently caused by phosphates in the sulfuric acid. Two other participants commented that the persulfate digestion consistently gave lower results than those obtained after mild acid hydrolysis. No satisfactory explanation was found for this phenomenon but it is probably due to incomplete decomposition of the persulfate during digestion as persulfate interferes with the colorimetry. One participant complained that difficulties are encountered with the stannous chloride method when samples are autoclaved; namely, after sulfuric-nitric acid or persulfate is added the final volume does not fall below the original sample volume, and hence, the sample cannot be neutralized before dilution to the original volume. Since it is difficult to believe that autoclaving at 15 to 25 psi for 30 minutes will fail to evaporate off at least 15 ml of water, it would appear that either the autoclave was faulty or that the samples were not autoclaved for the full 30 minutes.

SUMMARY AND CONCLUSIONS

Much dissatisfaction was found with the phenate method for ammonia nitrogen measurement, and the results indicate that nesslerization is the preferred method.

For measurement of nitrate nitrogen, the chromotropic acid, modified brucine (Jenkins and Medsker), and cadmium reduction methods are all very similar in performance and appear equally suitable for concentrations greater than 0.5 mg/liter. At 0.05 mg/liter, however, all three are unacceptably imprecise. The difficulty in obtaining sufficiently pure chromotropic acid and milled cadmium of the correct size and purity are considerations that give the preference to the modified brucine method. However, the AutoAnalyzer used with the hydrazine reduction method appears to be an excellent method but could not adequately be evaluated in this study, owing to insufficient data.

For the measurement of orthophosphates or polyphosphates after mild acid hydrolysis, the bismuth-catalyzed ammonium molybdate procedure appears satisfactory for concentrations greater than 0.1 mg/liter. For small concentrations, however, the stannous chloride procedure is the most precise.

The best results for measurement of total phosphate were obtained by the sulfuric-nitric acid digestion followed by the stannous chloride colorimetric procedure.

Much confusion exists over the interpretation of terms such as "polyphosphate," "acid-hydrolyzable phosphate," and "total phosphate," and about whether results should be expressed as P or PO_4. In some cases, a rewording of the instructions might be helpful, but the participant's habits and his failure to carefully read the instructions also played a part.

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C. Orthophosphate

1. Stannous Chloride Method. Appendix C.

2. Vanadomolybdophosphoric Acid Method. Appendix C.

3. Bismuth-Catalyzed Ortho and Polyphosphate Method for Phosphate - Treated Waters. Appendix D.

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7. Stannous Chloride Method with AutoAnalyzer.

8. Hach Chemical Co. Method. (Stannous Chloride).

9. Bismuth-Catalyzed Method (Appendix D), modified by use of Potassium Antimonyl Tartrate and Ascorbic Acid.

D. Polyphosphate

1. Mild Acid Hydrolysis with Stannous Chloride Method.

2. Mild Acid Hydrolysis with Vanadomolybdate.

3. Bismuth-Catalyzed Ortho and Polyphosphate Method for Phosphate-Treated Waters. Appendix D.

4. Mild Acid Hydrolysis with Murphy and Riley Reagent (orthophosphate reference 6).

5. AutoAnalyzer with Stannous Chloride.

6. Ammonium Molybdate with Ascorbic Acid.

7. Hach Chemical Co. Method (Stannous Chloride).

8. Bismuth-Catalyzed Method (Appendix D) with Potassium Antimonyl Tartrate.

E. Total Phosphate

1. Perchloric Acid Digestion with Stannous Chloride Method. Appendix C.

2. Perchloric Acid Digestion with Vanadomolybdate Method. Appendix C.

3. Sulfuric Acid - Nitric Acid Digestion with Stannous Chloride Method. Appendix C.

4. Sulfuric Acid - Nitric Acid Digestion with Vanadomolybdate Method. Appendix C.

5. Sulfuric Acid - Nitric Acid Digestion (Appendix C) with Bismuth-Catalyzed Method (Appendix D).

6. Sulfuric Acid - Nitric Acid Digestion (Appendix C) with Ammonium Molybdate and Ascorbic Acid (Orthophosphate reference 5).

7. Persulfate Digestion (Appendix C) with Bismuth-Catalyzed Method. Modified by use of Potassium Antimonyl Tartrate and Ascorbic Acid. (Orthophosphate reference 9).

8. Persulfate Digestion (Appendix C) with Bismuth-Catalyzed Method (Appendix D).

9. Persulfate Digestion with Stannous Chloride Method (Appendix C).

10. Persulfate Digestion with Vanadomolybdate Method (Appendix C).

11. Ammonium Persulfate Digestion with Stannous Chloride Method Using AutoAnalyzer.

12. Persulfate Digestion (Appendix C) with Aminonaphtholsulfonic Acid Method (Orthophosphate reference 4).

13. Persulfate Digestion (Appendix C) with Murphy and Riley Method (Orthophosphate reference 6).

14. Monoperoxysulfuric Acid Digestion with Stannous Chloride Method (Appendix C) Using AutoAnalyzer.

APPENDICES

APPENDIX A.

PHENATE METHOD FOR AMMONIA

1. GENERAL DISCUSSION

1.1. Principle: An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by a manganous salt.

1.2. Interference: Over 500 mg/liter, alkalinity interferes; over 100 mg/liter, acidity, color, and turbidity interfere. These interferences may be removed by preliminary distillation.

1.3. Absorptivity: 400.

2. APPARATUS

2.1. Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 630 mμ with a light path of approximately 1 cm.

b. Filter photometer, equipped with a red-orange filter having a maximum transmittance near 630 mμ and providing a light path of approximately 1 cm.

2.2. Magnetic stirrer.

3. REAGENTS

3.1. Ammonia-free water: Prepare as directed in Sec. 3.1 under Preliminary Distillation. (SMEWW, 12th edition, p. 189)

3.2. Hypochlorous acid reagent: To 40 ml distilled water add 10 ml 5 per cent commercial bleach. Adjust pH to 6.5 to 7.0 with HCl. This reagent is not stable and should be prepared fresh weekly.

3.3. Manganous sulfate solution, 0.003 M: Dissolve 50 mg $MnSO_4 \cdot H_2O$ in 100 ml distilled water.

3.4. Phenate reagent: Dissolve 2.5 g NaOH and 10 g phenol in 100 ml water. This reagent darkens on standing and should be prepared fresh weekly.

3.5. Stock ammonia solution: Dissolve 382 mg anhydrous ammonium chloride, NH_4Cl, dried at 100° C, in ammonia-free water and dilute to 1,000 ml. This solution has a concentration of 100 mg/liter ammonia nitrogen.

3.6. Standard ammonia solution: Dilute 5.00 ml stock ammonia solution to 1,000 ml with ammonia-free water; 1.00 ml = 0.50 μg N.

4. PROCEDURE

To a 10.0-ml sample in a 50-ml beaker, add 1 drop (0.05 ml) manganous sulfate solution. Place on a magnetic stirrer, and add 0.5 ml hypochlorous acid solution, followed immediately by the addition, a drop at a time, of 0.6 ml phenate reagent. Avoid erratically low results by adding the phenate reagent without appreciable delay and yet not too rapidly. Use bulb pipets for convenient delivery of the reagents. Mark the pipet for hypochlorous acid at the 0.5-ml level, and deliver the phenate reagent from a pipet which has been calibrated by counting the number of drops previously found to be equivalent to 0.6 ml. Stir vigorously during the addition of the reagents. Since color intensity is affected somewhat by the age of the reagents, carry a blank and a standard through the procedure along with each batch of unknowns. Measure the color after zeroing the photometer on the blank. Color formation is complete in 10 min and is stable for at least 24 hr. Although the blue color has a maximum absorbance at 630 mμ, satisfactory measurements can be made in the 600 to 660 mμ range.

5. CALCULATION

Beer's law governs. Calculate the ammonia concentration as follows:

$$\text{mg } NH_3\text{-N/l} = \frac{A \times B}{C}$$

in which A is absorbance of sample, B is concentration of standard, and C is absorbance of standard.

6. PRECISION AND ACCURACY

This method has a sensitivity of 10 μg/liter. Precision and accuracy are both approximately the same as for nesslerization.

APPENDIX B.

NITRATE (NITROGEN) METHODS

Nitrate represents the most highly oxidized phase in the nitrogen cycle and normally reaches important concentrations in the final stages of biologic oxidation. It generally occurs in trace quantities in surface water supplies but may attain large concentrations in some ground waters. In excessive amounts, it contributes to the illness known as infant methemoglobinemia. A limit of 45 mg NO_3/liter has accordingly been imposed on drinking waters as a means of averting this condition. The nitrate concentration of most drinking waters is usually less than 10 mg NO_3/liter.

Selection of method. Nitrate is a difficult determination in the presence of interfering ions. Three nitrate methods are presented for three different concentration ranges. These are: Method A, the cadmium reduction method for concentrations less than 0.1 mg NO_3-N/liter; Method B, the brucine method with salt masking for concentrations between 0.1 and 2 mg NO_3-N/liter; Method C, the chromotropic acid method for concentrations greater than 2 mg NO_3-N/liter. The ranges of these methods are not rigid and are to be used mainly as guidelines.

Storage of sample. To prevent any change in the nitrogen balance through biologic activity, the nitrate determinations should be started promptly after sampling. If such a step is impractical, storage near freezing either in the presence of preservatives such as H_2SO_4 (0.8 ml conc H_2SO_4/liter) or $HgCl_2$ (40 mg Hg^{++}/liter) or without preservative is recommended. If acid preservation is employed, it is important that the sample's acidity be neutralized to at least pH 7 immediately before the procedure is undertaken.

A. CADMIUM REDUCTION METHOD (TENTATIVE)

1. GENERAL DISCUSSION

Nitrate is reduced almost quantitatively to nitrite when a sample is run through a column containing amalgamated cadmium filings. The nitrite thus produced is determined by diazotizing with sulfanilamide and coupling with N-(1-naphthyl)-ethylenediamine to form a highly colored azo dye which is measured colorimetrically. A correction may be made for any nitrite initially present in the sample.

2. APPARATUS

2.1. Reduction columns (see Figure B-1): Constructed from three pieces of glass tubing joined end to end, viz., 10 cm of 5-cm i. d. tube onto 30 cm of tubing 10 mm i. d., which in turn is joined to 35 cm of 2-mm i. d. tube. The last tube is bent just below this joint into a U so

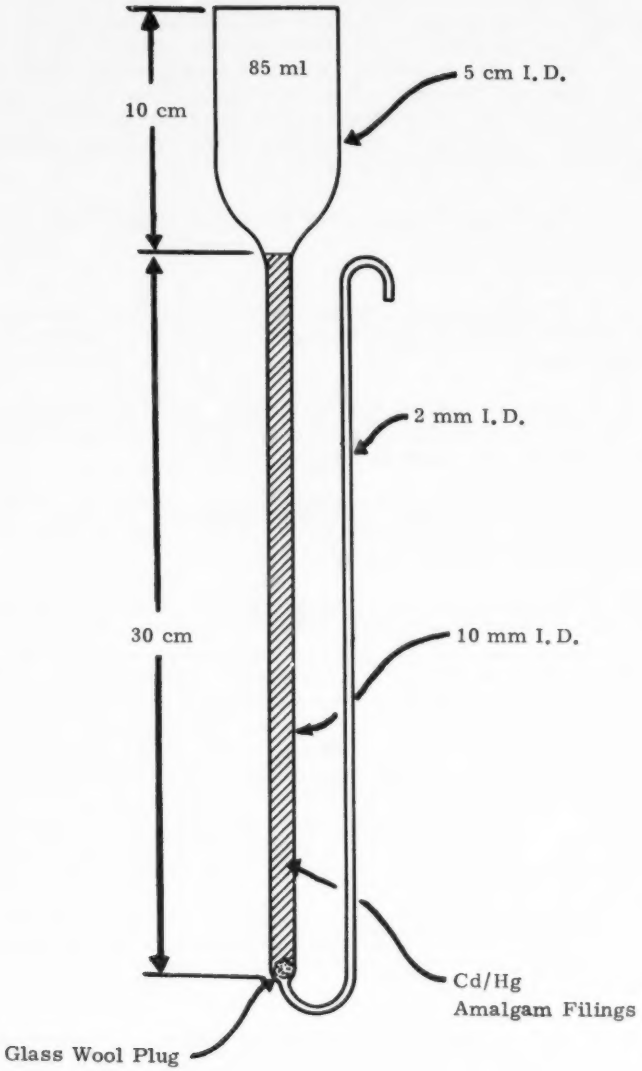

Figure B-1. Reduction Column

that it runs up parallel to the 10-mm-diameter tube and then its end is bent over to form an inverted U siphon. This last bend should be just level with the top of the 10-mm-diameter tube when the assembly is held upright to form a column. With this arrangement, liquid placed in the top reservoir tube should flow out of the system and stop when the level of liquid is such that it just covers the metal filings (see below). Place a mark on the upper wide portion of the column to indicate the height of an additional 80 ml of water.

2.2. Spectrophotometer, capable of reading at 543 mμ.

3. REAGENTS

3.1. Distilled water: Distilled water should be of the highest purity, preferably prepared by mixed-bed ion exchange deionization of ordinary distilled water.

3.2. Concentrated ammonium chloride solution: Dissolve 100 g of analytical-reagent-quality ammonium chloride in 500 ml of distilled water and store in a glass or plastic bottle.

3.3. Dilute ammonium chloride solution: Dilute 50 ml of concentrated ammonium chloride solution to 2,000 ml with distilled water. Store the solution in a glass or plastic bottle.

3.4. Amalgamated cadmium filings: File sticks of pure cadmium metal (reagent grade) with a coarse metal hand file (about second cut) and collect the fraction that passes a sieve with 2-mm openings and is retained on a sieve with 0.5-mm openings. Stir about 300 g of filings with 300 ml of a 1 percent by weight solution of mercuric chloride for 3 min. (This amount should be sufficient for six reduction columns.) Allow the metal particles to settle and decant off the mercury solution. Wash the amalgamated filings several times with distilled water. Wash the cadmium filings briefly in 1 percent v/v nitric acid and then a few times with dilute (1 percent) hydrochloric acid. Wash copiously with distilled water until no nitrite can be detected in the supernatant fluid. Store the filings in the dark under dilute ammonium chloride solution.

3.5. Sulfanilamide solution: Dissolve 5 g of sulfanilamide in a mixture of 50 ml of conc hydrochloric acid (s.g. 1.18) and about 300 ml of distilled water. Dilute to 500 ml with water. The solution is stable for many months.

3.6. N-(1-naphthyl)-ethylenediamine dihydrochloride solution: Dissolve 0.50 g of the dihydrochloride in 500 ml distilled water. Store the solution in a dark bottle. The solution should be renewed once a month or immediately when it develops a strong brown coloration.

3.7. Stock standard nitrate solution: Dissolve 0.722 g dry reagent-grade KNO_3 in distilled water and dilute to 1 liter.

3.8. Dilute standard nitrate solution: Dilute 4 ml stock standard nitrate to 2 liters with distilled water and use immediately 1 ml = 0.2 μg NO_3-N.

4. PROCEDURE

4.1. Preparation of reduction columns: Ram a plug of glass wool into the bottom of a reductor column and fill it with distilled water. Pour into it sufficient cadmium filings to produce a column 30 cm in length. The size of the reductor column is important since columns of smaller diameter and length give erratic results and show rapid deterioration. Wash the column thoroughly with dilute ammonium chloride solution. The flow rate should be such that 80 ml of solution added to the container takes not less than 10 to 12 min to flow through the column. If the rate is too fast it may be slowed by constricting the end of the outlet siphon or by packing more glass wool at the base of the column. Periods in excess of about 15 min unnecessarily increase the time for an analysis and may give low results. When not in use columns should be left with the metal covered by dilute ammonium chloride solution.

4.2. Nitrate analysis: If turbidity or suspended solids are present the sample should be clarified by membrane filtration.

4.3. Adjust the pH of the sample to within the range of pH 8 to 9 if more alkaline than this by using a pH meter and dilute HCl.

4.4. To an 80- to 90-ml sample in a 125-ml erlenmeyer flask add 2.0 ml conc ammonium chloride solution. Mix the solutions and pour the sample from the erlenmeyer flask onto the column until the marked level in the top part of the column is reached. Place a 50-ml graduated cylinder under the siphon to collect the effluent and discard any sample left in the erlenmeyer flask. Shake the flask as dry as possible and retain it for collecting the main portion of the effluent.

4.5. Allow between 25 and 30 ml of effluent to collect in the cylinder and then replace it by the original erlenmeyer flask which had contained the sample. Discard the contents of the cylinder. The passage of 25 to 30 ml of solution removes liquid (ammonium chloride solution or a previous sample) from the dead space of the reduction column. The volume flushed through is not critical provided it exceeds 25 ml, but sufficient sample should be left in the column so that 50 ml more can be reduced. The flushing effluent should therefore not exceed about 30 ml. A maximum of about 8 columns can be handled conveniently at one time. The operator should experiment to find a suitable short delay between adding

the samples to each column so that there will be time to reject the flushing liquid from one column and replace the cylinder by the erlenmeyer flask before too much flushing liquid has escaped from the next column in line. When a sample having a very small concentration of nitrate is followed by a sample with a large nitrate concentration (or vice versa) a full 30 ml should be used to flush the column.

4.6. Allow the remainder of the sample to collect in the flask and when the flow of sample from the column has ceased pour exactly 50 ml of solution from the flask into the 50-ml measuring cylinder. Drain and discard the remaining effluent from the erlenmeyer flask. Shake the flask as dry as possible and then pour back into it the 50 ml of reduced sample from the measuring cylinder.

4.7. There is no need to wash columns between samples, but if columns are not to be reused for several hours or longer, 50 ml of dilute ammonium chloride solution should be poured onto the top and allowed to pass through the system. The cadmium filings are then stored in this ammonium chloride solution and should never be allowed to go dry. If there are indications of the columns' becoming deactivated, as will be shown by a significant decrease in the color intensity produced per μg NO_3-N in NO_3-N standards then the columns must be emptied, the cadmium filings washed briefly with 1 percent v/v nitric acid and copiously with distilled water, air dried at about 60°C, and resieved and reamalgamated. Well-prepared columns of the correct size should be stable for many weeks before repreparation becomes necessary.

4.8. As soon as possible after reduction and in any case no longer than 15 min after add 1.0 ml of sulfanilamide solution from an automatic pipette. Allow the reagent to react for a period greater than 2 min but not exceeding 8 min. Add 1.0 ml of α-naphthylethylenediamine solution and mix immediately. Between 10 min and 2 hr afterwards measure the extinction of the solution against a distilled-water reagent blank by using a wavelength of 543 mμ. As a guideline the following light paths are recommended for the indicated NO_3-N concentration ranges:

Light path length, cm	NO_3-N concentration, mg/liter
1	0.02 to 0.20
5	0.02 to 0.06
10	<0.02

4.9. Standards: The purpose of running standards in this determination is twofold: First to obtain a standard curve and second to check the efficiency of the Cd reduction columns.

Add about 110 ml dilute standard nitrate solution to clean, dry 125-ml erlenmeyer flasks and carry out the determination exactly as described for samples, except flush the columns first with 50 ml of standard. Measure the absorbance in a 1-cm cell. Perform the experiment initially in triplicate for each column and correct the mean of the three absorbances thus obtained by the absorbance of a distilled-water reagent blank. Subsequent daily check need only be in the form of one determination for each column.

Determine the factor F (relating absorbance in a 1-cm cell to NO_3-N concentration, mg/liter) from the following equation:

$$F = \frac{0.2}{A}$$

where A is the absorbance at 543 mμ.

For columns with satisfactory reducing efficiency the value of F should be in the region of 0.33. A consistent increase in the value of F indicates a column of decreasing efficiency and that reactivation may be necessary.

5. CALCULATION

$$\text{mg } NO_3\text{-N/liter} = [A_S - A_B] \times \frac{F}{L} - C$$

when A_S = absorbance of sample

A_B = absorbance of reagent blank

$$F = \frac{0.2}{\text{Absorbance of 0.2 mg } NO_3\text{-N/liter standard in 1-cm path length cell}}$$

L = light path length

C = concentration of NO_2-N, mg/liter

B. BRUCINE METHOD (TENTATIVE)

1. GENERAL DISCUSSION

The reaction between nitrate and brucine produces a yellow color which can be used for the colorimetric estimation of nitrate. The intensity of the color is measured at 410 mμ. The reaction rate between brucine and nitrate ion is affected significantly by the amount of heat generated during the test, and thus the procedure seeks heat control by reagent addition sequence and incubation of the reaction mixture for a precise interval of time at a known temperature. Acid concentration

and reaction time have been selected to yield optimum development and stability of color. The method works well in waters of salinities varying from that of fresh water to that of sea water. The method is recommended for the approximate range of 0.1 to 2 mg NO_3-N/liter.

Interferences. All strong oxidizing or reducing agents interfere. The presence of oxidizing agents may be determined by the addition of orthotolidine reagent as in the measurement of residual chlorine. The interference by residual chlorine may be eliminated by the addition of sodium arsenite, provided that the residual chlorine does not exceed 5 mg/liter. A slight excess of sodium arsenite will not affect the determination. Ferrous and ferric iron and quadrivalent manganese give slight positive interferences, but in concentrations less than 1 mg/liter these are negligible. The interference due to nitrite up to 0.5 mg NO_2-N/liter is eliminated by the use of sulfanilic acid. Chloride interference is masked by the addition of excess NaCl.

Large concentrations of organic matter such as in undiluted raw wastewater will usually interfere.

2. APPARATUS

2.1. Colorimetric equipment: One of the following is required:

a. Spectrophotometer, for use at 410 mμ providing a light path of 1 inch.

b. Filter photometer, providing a light path of 1 inch and equipped with a violet filter having maximum transmittance of from 400 to 425 mμ.

2.2. Safety pipette.

2.3. Wire racks, to hold tubes in which samples are to be incubated (Van Waters and Rogers No. 60935 or equivalent).

2.4. Stirred boiling water bath, with sufficient heating facility to maintain a temperature of at least 95°C when cooled samples are introduced.

2.5. Reaction tubes: Hard glass test tubes of approximate dimensions 2.5 × 15 cm in which reaction is performed. (The 1-inch colorimeter tubes [Van Waters and Rogers No. 22366] used in conjunction with the Bausch and Lomb Spectronic 20 or equivalent have been used for convenience since their use avoids the necessity for a transfer following reaction, to determine transmittance.)

3. REAGENTS

3.1. Stock nitrate solution: Dissolve 0.7218 g anhydrous potassium nitrate, KNO_3, and dilute to 1,000 ml with distilled water. This solution contains 100 mg/liter N.

3.2. Standard nitrate solution: Dilute 10.0 ml stock nitrate solution to 1,000 ml with distilled water; 1.00 ml = 1.0 μg N. Prepare immediately prior to using.

3.3. Sodium arsenite solution: Dissolve 5.0 g $NaAsO_2$ and dilute to 1 liter with distilled water. (CAUTION: Toxic; take care to avoid ingestion.)

3.4. Brucine-sulfanilic acid solution: Dissolve 1 g brucine sulfate and 0.1 g sulfanilic acid in approximately 70 ml hot distilled water. Add 3 ml conc HCl, cool, and make up to 100 ml. This solution is stable for several months. The pink color that develops slowly does not affect its usefulness. (CAUTION: Brucine is toxic; take care to avoid ingestion.)

3.5. Sulfuric acid solution: Carefully add 500 ml conc H_2SO_4 to 125 ml distilled water. Cool to room temperature before using and keep tightly stoppered to prevent absorption of atmospheric moisture.

3.6. Sodium chloride solution, 30 percent: Dissolve 300 g NaCl and dilute to 1,000 ml with distilled water.

4. PROCEDURE

4.1. Pretreatment of sample: If the sample contains chlorine, remove it by adding 1 drop (0.05 ml) sodium arsenite solution for each 0.10 mg Cl, and mix. Add 1 drop in excess to a 50-ml portion.

4.2. Color development: Set up the required number of reaction tubes in the wire rack, spacing them so that each tube is surrounded by empty spaces. Include a reaction tube for a reagent blank and reaction tubes for as many standards as desired. To each tube add 10 ml of sample or an aliquot diluted to 10 ml so that the sample volume taken for analysis contains 0.1 to 8 μg NO_3-N. Place the rack in a cool water bath and add 2 ml of 30 percent NaCl solution. Mix thoroughly by swirling and add 10 ml H_2SO_4 solution. Although thorough mixing is essential this should be accomplished by swirling by hand. In no case should a Vortex mixer be used since this type of mixing produces inconsistent results in the analysis. Mix again thoroughly by swirling and allow to cool. At this point, if any turbidity or color is present or if optically unmatched colorimeter tubes are being used as reaction tubes, the tubes should be dried off and a "sample blank" value read against the reagent

blank tube at 410 mμ. Replace the rack of tubes in the cool water bath and add 0.5 ml brucine-sulfanilic acid reagent. Swirl the tubes to mix thoroughly and then place the rack of tubes in a well-stirred boiling water bath that maintains a temperature of not less than 95° C. After exactly 20 min, remove the samples and immerse them in a cold water bath. When thermal equilibrium is reached (at approx room temperature), dry off the tubes with tissue and read the standards and samples against the reagent blank at 410 mμ in the spectrophotometer. Note: To provide a check on technique and the constancy of reaction conditions, it is wise to run at least two standards with each batch of samples.

5. CALCULATION

To obtain a standard curve, subtract the "sample blanks" from the final absorbance readings and plot the resultant absorbance densities against mg NO_3-N/liter. Corrected absorbance readings for the samples are obtained by subtracting their "sample blank" values from their final absorbance values. Concentrations of NO_3-N may be determined by reading directly from the standard curve:

$$\text{mg/liter nitrate N} = \frac{\mu\text{g } NO_3\text{-N}}{\text{ml sample}}$$

$$\text{mg/liter } NO_3 = \text{mg/liter nitrate N} \times 4.43$$

C. CHROMOTROPIC ACID METHOD (TENTATIVE)

1. GENERAL DISCUSSION

1.1. Principle: Two moles of nitrate react with 1 mole of chromotropic acid to form a yellow reaction product exhibiting greatest absorbance at 410 mμ. The maximum color develops within 10 minutes and is stable for 24 hours. The cooling bath dissipates sufficient heat to prevent the boiling of the solutions and the formation of bubbles. For this reason, the temperature range of the cooling bath may vary from 10° C to 20° C without critically affecting the results. Residual chlorine, certain oxidants, and nitrite also yield yellow colors with chromotropic acid. However, the addition of sulfite completely eliminates the interference from residual chlorine and oxidizing agents, while urea deactivates the nitrite through conversion to nitrogen gas. Antimony effectively masks up to 2,000 mg/liter chloride, a tolerance level which can be raised to 4,000 mg/liter by doubling the strength of the specified antimony reagent.

1.2. Interference: The yellow color of the chloroferrate (III) complex in amounts up to 40 mg/liter ferric ion is completely discharged through demasking by the addition of antimony. Barium, lead, strontium,

iodide, iodate, selenite, and selenate ions are incompatible with the system and form precipitates. However, the unlikelihood of their presence in significant amounts in ordinary waters offers no complications. Concentrations exceeding 20 mg/liter chromic ion interfere with the absorbance of its sulfate complex.

1.3. Minimum detectable concentration: 50 μg/liter nitrate N.

1.4. Absorptivity.

2. APPARATUS

Colorimetric equipment: One of the following is required.

2.1. Spectrophotometer, for use at 410 mμ providing a light path of 1 cm or longer.

2.2. Filter photometer, providing a light path of 1 cm or longer and equipped with a violet filter having maximum transmittance near 410 mμ.

2. REAGENTS

3.1. Redistilled water: Redistill single-distilled water from an all-pyrex still. Prepare all-aqueous solutions from this double-distilled water.

3.2. Stock nitrate solution: Dissolve 721.8 mg anhydrous potassium nitrate, KNO_3, in redistilled water and dilute to 1,000 ml; 1.00 ml = 100 μg N.

3.3. Standard nitrate solution: Dilute 100.0 ml stock nitrate solution to 1,000 ml with redistilled water; 1.00 ml = 10.0 μg N.

3.4. Sulfite-urea reagent: Dissolve 5 g urea and 4 g anhydrous sodium sulfite, Na_2SO_3, in redistilled water and dilute to 100 ml.

3.5. Antimony reagent: Heat 500 mg antimony metal in 80 ml conc sulfuric acid until all of the metal has dissolved. Cool, and cautiously add to 20 ml iced, redistilled water. Redissolve by heating any salt which crystallizes after standing overnight.

3.6. Chromotropic acid reagent: Purify the chromotropic acid (4, 5-dihydroxy-2, 7-naphthalene disulfonic acid) in the following manner. Boil 125 ml distilled water in a beaker and gradually add 15 g 4, 5-dihydroxy-2, 7-naphthalene disulfonic acid disodium salt with constant stirring. To the solution add 5 g activated decolorizing charcoal. Boil the mixture for

about 10 min. Add distilled water to make up the loss due to evaporation. Filter the hot solution through cotton woll plugged in the funnel. Add 5 g activated charcoal to the filtrate and boil for 10 more min. First filter through cotton wool and then filter through a filter paper to remove the charcoal completely. Cool the solution and slowly add 10 ml nitrate-free conc sulfuric acid. Boil the solution until about 100 ml is left in the beaker. Allow the solution to stand overnight. Transfer the crystals of chromotropic acid to a Buchner funnel and wash thoroughly with 95 percent alcohol until the crystals are white. Dry the crystals at 80°C.

Dissolve 100 mg purified chromotropic acid in 100 ml conc sulfuric acid and store in a brown bottle. Prepare every 2 weeks. A colorless reagent solution signifies the absence of nitrate contamination from the sulfuric acid.

3.7. Sulfuric acid, conc, nitrate-free.

4. PROCEDURE

4.1. Preparation of nitrate standards: Prepare nitrate standards in the range 0.10 to 5 mg/liter N by diluting appropriate volumes of the standard nitrate solution to 100 ml with redistilled water. Suggested volumes of standard nitrate solution are 0, 1.0, 5.0, 15, 25, 35, and 50 ml.

4.2. Color development: Pipette 2.5 ml nitrate standards and clear sample in the nitrogen range of 0.05 to 5 mg/liter (0.125 to 12.5 μg), together with a reagent blank prepared from redistilled water, into dry 10-ml volumetric flasks. To each flask add 1 drop (0.05 ml) sulfite-urea reagent, place the flasks in a tray of cold water (10°C to 20°C) and add 2 ml antimony reagent. Swirl the flasks during the addition of each reagent. After the mixtures have stood in the bath for about 4 min, add 1 ml chromotropic acid reagent, swirl the flasks again, and then allow to stand in the cooling bath for an additional 3 min. Next add conc sulfuric acid to adjust the volume to the 10-ml mark, stopper the flasks, and mix the contents by inverting them four times. Finally, allow the solutions to stand for 45 min at room temperature and again adjust the volume to the 10-ml mark with conc sulfuric acid. Perform the final mixing very gently so as not to introduce gas bubbles. Read the absorbance at 410 mμ by using water in the reference cell 15 min or more after the last adjustment of volume. Rinse the cell with the solution and then fill carefully so as to avoid trapping bubbles. For this operation keep the cell in a slanting position (30° to horizontal) and pour the solution very slowly down the side of the cell.

Remove appreciable amounts of suspended matter in the sample by centrifugation or filtration.

5. CALCULATION

$$\text{mg/liter nitrate N} = \frac{\mu\text{g nitrate N}}{\text{ml sample}}$$

$$\text{mg/liter } NO_3 = \text{mg/liter nitrate N} \times 4.43$$

6. PRECISION AND ACCURACY

A synthetic unknown sample containing 1.0 mg/liter nitrate N, 10 mg/liter chloride, 200 µg/liter ammonia N, 1.5 mg/liter organic N, 10.0 mg/liter phosphate, and 5.0 mg/liter silica in distilled water was determined by the chromotropic acid method with a relative standard deviation of 8.1 percent and a relative error of 3.0 percent in five laboratories.

A second synthetic unknown sample containing 1.0 mg/liter nitrate N, 200 mg/liter chloride, 800 µg/liter ammonia N, 800 µg/liter organic N, 5.0 mg/liter phosphate, and 15.0 mg/liter silica in distilled water was determined by the chromotropic acid method with a relative standard deviation of 1.2 percent and no relative error in four laboratories.

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APPENDIX C.

ORTHO, POLY, AND TOTAL PHOSPHATE (PHOSPHORUS) METHODS FOR NATURAL AND TREATED WATERS

INTRODUCTION

Phosphorus occurs in natural waters and in waste waters almost solely in the form of various types of phosphate. These forms are commonly classified into orthophosphates, condensed phosphates (pyro-, meta-, poly-phosphates) and organically bound phosphates. These forms of phosphate may occur in the soluble form, in particles of detritus, or in the bodies of aquatic organisms.

Phosphorus is an element which is essential to the growth of organisms and can often be the nutrient that limits the growth that a body of water can support. In instances where phosphorus is a growth-limiting nutrient, the discharge of raw or treated sewage, agricultural drainage, and certain industrial wastes to a receiving water may stimulate the growth of nuisance quantities of photosynthetic aquatic micro- and macro-organisms.

Phosphates occur in bottom sediments and in biological sludges both as precipitated inorganic forms and incorporated into organic compounds.

METHODS OF ANALYSIS

Phosphate analyses consist of two general procedural steps: (1) conversion of the phosphorus form of interest to soluble orthophosphate and (2) colorimetric determination of soluble orthophosphate. The separation of phosphorus into its various forms is largely analytically defined, but the analytical differentiations have been selected so that they may be used for interpretive purposes.

The analytical scheme for phosphate differentiation can be summarized as follows:

1. Total phosphate: Digestion of whole sample followed by orthophosphate determination.

2. Total filterable (soluble) phosphate: Filtration of whole sample through 0.45 μ membrane filter followed by digestion of filtrate and by orthophosphate determination.

3. Filterable (soluble) acid-hydrolyzable phosphate: Filtration of whole sample through 0.45 μ membrane filter followed by mild acid hydrolysis of filtrate and orthophosphate determination.

4. Total orthophosphate: Orthophosphate determination on whole sample.

5. Filterable (soluble) orthophosphate: Filtration of whole sample through 0.45 μ membrane filter followed by orthophosphate determination on the filtrate.

DISCUSSION

Digestion techniques

Since phosphorus may occur in suspension and in combination with organic matter a digestion method to determine total phosphate must be able to oxidize organic matter effectively, rupturing both C-P and C-O-P bonds, and to solubilize suspended material to release the phosphorus as soluble orthophosphate.

Three digestion methods are given. The perchloric acid method is the most drastic and time-consuming method and is recommended only for particularly difficult samples such as sediments. The nitric acid/sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technique. It is recommended that this method be checked against one or more of the more drastic digestion techniques and be adopted if identical recoveries are obtained.

Filtration techniques

Separation of "soluble" and "particulate" forms of phosphate is made by using filtration through a 0.45 μ membrane filter. Membrane filtration is selected over depth filtration because of the greater likelihood of obtaining a consistent separation of particle sizes by the membrane filtration technique. No claim is made that filtration through 0.45 μ membrane filters is a true separation of suspended and soluble forms of phosphate; it is merely a convenient and replicable analytical technique designed to make a gross separation. This is reflected in the use of the term "filterable" (rather than "soluble") to describe the phosphorus forms determined after 0.45 μ membrane filtration.

Acid hydrolysis

Acid hydrolysis at boiling water temperatures is designed to convert filterable and particulate condensed phosphates to filterable orthophosphate. The hydrolysis unavoidably releases some phosphate from organic

compounds, but this factor has been reduced to a minimum (consistent with good condensed phosphate hydrolysis) by judicious selection of acid strength and hydrolysis time and temperature. Nevertheless, the term "acid hydrolyzable phosphate," rather than "condensed phosphate," is preferred for this technique.

Colorimetric determination of orthophosphate

Two methods of orthophosphate determination are given. The selection of the method depends largely on the concentration range of orthophosphate. Thus the vanadomolybdic acid method is most useful for routine analyses in the range of 0.2 to 18 mg P/liter. The stannous chloride method is more suited for the range of 0.01 to 6 mg P/liter. An extraction step is recommended for the lower part of this range and when interferences need to be overcome.

Sampling and storage: If a differentiation of phosphorus forms is to be made it is recommended that filtration be carried out immediately upon sample collection and that samples be preserved by freezing (at or below -10°C) either in the presence or absence of 40 mg $HgCl_2$/liter. The addition of the mercury preservative is specially recommended if samples are to be stored for long periods. Preservation with acid or chloroform should be avoided. If total phosphorus alone is to be analyzed, preservation is not necessary. Samples containing small concentrations of phosphorus should not be stored in plastic bottles, because it is suspected that sorption of phosphate onto the walls of the bottles takes place. All glass containers should be rinsed with hot, dilute HCl followed by several rinses of distilled water. Commercial detergents containing phosphate should not be used for cleansing the glassware used in phosphate analyses.

FILTERABLE ORTHOPHOSPHATE DETERMINATION

A. VANADOMOLYBDOPHOSPHORIC ACID METHOD

1. GENERAL DISCUSSION

1.1. Principle: In a dilute orthophosphate solution, ammonium molybdate reacts in an acid solution to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium the vanadomolybdophosphoric yellow color is formed. The intensity of yellow color is proportional to phosphate concentration in solution.

1.2. Interference: Positive interference is caused by silica and arsenic only if the sample is heated. Negative interferences are caused by arsenate, fluoride, Th, Bi, sulfide, thiosulfate, thiocyanate, or excess molybdate. Blue color is caused by ferrous iron, but this does

not affect results if the Fe^{2+} is less than 100 mg/liter. Sulfide interferences may be removed by oxidation with bromine water. Ions that do not interfere in concentrations up to 1,000 mg/liter are Al, Fe^{3+}, Mg, Ca, Ba, Sr, Li, Na, K, NH_4^+, Cd, Mn, Pb, Hg^+, Hg^{++}, Sn^{++}, Cu, and Ni, (by change in hue), Ag, U, Zr, arsenite, Br, $CO_3^=$, ClO_4^-, CN^-, IO_3^-, SiO_4, NO_3^-, NO_2^-, $SO_4^=$, $SO_3^=$, pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, and salicylate. If nitric acid is used in the test, chloride interferes at 75 mg/liter.

1.3. Minimum detectable concentration: The minimum detectable concentration is 0.2 mg/liter in 1-cm spectrophotometer cells.

2. APPARATUS

2.1. Colorimetric equipment: Visual comparison in nessler tubes is not recommended, because the sensitivity of the method is dependent on wavelength used. One of the following is required:

a. Spectrophotometer, for use at approximately 400 to 490 mμ.

b. Filter photometer, provided with a filter with maximum transmission between 400 and 490 mμ.

The wavelength at which color intensity is measured depends on the sensitivity desired since the sensitivity varies tenfold with wavelengths from 400 to 490 mμ. Ferric iron causes interference at low wavelengths, particularly at 400 mμ. A wavelength of 470 mμ is generally employed. Concentration ranges for different wavelengths are:

Range, mg/liter P	Wavelength, mμ
0.75 to 5.5	400
2.0 to 15	440
4 to 17	470
7 to 20	490

2.2. Filtration equipment: See Residue, Method B, Section 2.1a., p. 245, SMEWW, 12th edition. Membrane filters should be washed by soaking in distilled water prior to use in separations since they may contribute significant amounts of phosphorus to samples containing small concentrations of phosphate. Two washing techniques are suggested: (a) Soak filters (50 per 2 liters) in distilled water for 24 hr (b) soak filters (50 per 2 liters) in distilled water for 1 hr., change the distilled water and soak filters in distilled water for 3 hr.

2.3. Acid-washed glassware: This may be of great importance, particularly in determining small concentrations of phosphate. Phosphate contamination is common owing to adsorption on glass surfaces. Commercial detergents containing phosphate should be avoided. Glassware should be cleansed with hot, dilute HCl and rinsed well with distilled water.

3. REAGENTS

3.1. Phenolphthalein indicator solution: Either aqueous (a) or alcoholic (b) solution may be used.

a. Dissolve 5 g phenolphthalein disodium salt in distilled water and dilute to 1 liter. If necessary, add 0.02 N NaOH dropwise until a faint pink color appears.

b. Dissolve 5 g phenolphthalein in 500 ml of 95 percent ethyl alcohol or isopropyl alcohol and add 500 ml distilled water. Then add 0.02 N NaOH until a faint pink color appears.

3.2. Vanadate-molybdate reagent:

a. Solution A. Dissolve 25 g ammonium molybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 400 ml distilled water.

b. Solution B. Dissolve 1.25 g of ammonium metavanadate, NH_4VO_3, in 300 ml of boiling distilled water. Cool and add 330 ml concentrated HCl. Cool solution to room temperature and pour solution A into solution B and dilute the mixture to 1 liter.

Note: H_2SO_4, $HClO_4$, or HNO_3 may be substituted for HCl. The acid concentration in the determination is not critical, but a final concentration of 0.5 N is recommended.

3.3. Standard phosphate solution: Dissolve in distilled water 0.2195 g anhydrous potassium dihydrogen phosphate, KH_2PO_4, and dilute to 1 liter.

$$1 \text{ ml} = 0.05 \text{ mg } PO_4 - P$$

4. PROCEDURE

4.1. Filter a portion of the sample through 0.5 ± 0.1 μ pore size membrane filters, avoiding possible contamination as explained above (see "sampling and storage" and Sec. 2.3). If filterable condensed phosphate concentration is to be determined, collect as much as 200 ml of filtrate and save the filtered and unfiltered samples for use in the filterable condensed phosphate determination. If the pH of the sample is less

than 4, dilute 50 ml to 100 ml in a volumetric flask with distilled water and mix thoroughly. Use this diluted sample in the following steps. If the pH is greater than 10, add 1 drop of phenolphthalein indicator to 50.0 ml of sample and discharge the red color with concentrated HCl before diluting to 100 ml. (Such a dilution is also useful when concentrations greater than 15 mg PO_4-P/liter are present. When dilutions are made, the correct interpretation of "ml sample" in Sec. 5 must be made. For example, when 50 ml of original sample has been diluted to 100 ml for pH adjustment, the "ml sample" in the calculation is 25, not 50, although 50 ml of diluted sample is still used in the following steps.)

If excessive color is present in the sample it should be removed by shaking about 50 ml of sample with 200 mg of activated carbon (Darco G60 or equivalent quality) in an erlenmeyer flask for 5 min followed by filtering the sample through filter paper (Whatman No. 42 or equivalent) to remove carbon. Each batch of carbon should be checked for phosphate since some produce high reagent blanks.

4.2. Place 35 ml or less of the sample that contains 0.05 to 1.0 mg P in a 50-ml volumetric flask. Add 10 ml vanadate-molybdate reagent and dilute to the mark with distilled water. Prepare a blank in which 35 ml of distilled water is substituted for the sample solution. After 10 minutes from the time of addition of vanadate molybdate reagent, measure the percent transmittance of the sample versus a blank at a wavelength of 400 to 490 mμ dependent on sensitivity desired (see Sec. 2.1.). The color is stable for days and its intensity is unaffected by variations in room temperature.

4.3. A calibration curve is prepared by using a suitable volume of standard phosphate solution and distilled water in place of the sample and proceeding as in Sec. 4.2. When ferric ion is low enough not to interfere, a convenient practice is to plot a family of calibration curves of one series of standard solutions for various wavelengths. This permits a wide latitude in concentrations in one series of determinations. At least one standard should be analyzed with each set of samples.

5. CALCULATION

$$\text{mg/liter P} = \frac{\text{mg P} \times 1{,}000}{\text{ml sample}}$$

6. PRECISION AND ACCURACY

The standard deviation of the method was determined for distilled water samples to be 0.02 mg P/liter at the 10 mg P/liter level, 0.004 mg P/liter at the 2 mg P/liter level, and 0.002 mg P/liter at the 1 mg P/liter level.

B. STANNOUS CHLORIDE METHOD

1. GENERAL DISCUSSION

1.1. Principle: The principle of this method involves the formation of molybdophosphoric acid, which is reduced to the intensely colored complex molybdenum blue by stannous chloride. This method greatly increases sensitivity and makes an extraction step feasible, increasing the reliability of the method at concentrations less than 0.1 mg P/liter and lessening interferences.

1.2. Interference: Arsenic and germanium must be absent. Sulfide must be removed by oxidation, and saturated bromine water may be used for this purpose. The soluble-iron content of the portion taken for analysis should not exceed 0.1 mg. Tannin, lignin, and hexavalent chromium (at concentrations normally encountered) will cause significant error only when the phosphate sought is less than 1 mg/liter. Soluble silicates do not interfere even at concentrations as large as 100 mg/liter SiO_2. The presence of large amounts of polyphosphate in the water can lead to slightly high orthophosphate values; however, in no case is it likely that this error will exceed 0.1 mg/liter. In testing for phosphate in highly saline waters, such as brines, low results may be obtained. If so, readings should be taken on successive dilutions until two such dilutions give essentially the same value. Color and turbidity also interfere, but this interference can often be avoided by employing an extraction step. Chromate and strong oxidizing agents, such as peroxide, bleach the blue color. Interference from nitrite (which also bleaches the blue color) can be overcome by adding 0.1 g sulfamic acid to the sample before adding the molybdate. Because of the very low PO_4 range, contamination is a problem. In general, the direct method should be used only if Method A does not provide adequate sensitivity. Extracting the heteropoly acid into an immiscible solvent before reduction greatly reduces the number of interferences; however, it does not remove interference from arsenic and germanium. Extraction also reduces the amount of polyphosphate hydrolysis in the test.

1.3. Minimum detectable concentration: The minimum detectable concentration is about 0.003 mg/liter P. The sensitivity at 50 percent transmittance is about 0.01 mg/liter for 1 percent change in transmittance.

2. APPARATUS

The same apparatus is required as for Method A, Sec. 2, except when the extraction step is employed, in which case a safety aspirator is required. The spectrophotometer will be used at 625 mμ in the measurement of the benzene-isobutanol extracts, and at 690 mμ for the aqueous

solutions. A wavelength of 650 mμ may be used for the aqueous solutions, with somewhat reduced sensitivity and precision, if the instrument available is not equipped to read at 690 mμ.

3. REAGENTS

3.1. Phenolphthalein indicator solution: Prepare as directed in Method A, Sec. 3.1.

3.2. Strong-acid solution: Slowly add 300 ml conc H_2SO_4 to about 600 ml distilled water. When cool add 4.0 ml conc HNO_3 and dilute to 1 liter.

3.3. Ammonium molybdate reagent (I): Dissolve 25 g $(NH_4)_6Mo_7O_{24}$ $4H_2O$ in 175 ml distilled water. Cautiously add 280 ml conc H_2SO_4 to 400 ml distilled water. Cool, add the molybdate solution, and dilute to 1 liter.

3.4. Stannous chloride reagent (I): Dissolve 2.5 g of a fresh supply of $SnCl_2 \cdot 2H_2O$ in 100 ml glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.

3.5. Standard phosphate solution: Prepare as directed in Method A, Sec. 3.3.

3.6. Reagents for extraction:

a. Benzene-isobutanol solvent: Mix equal volumes of benzene and isobutyl alcohol. (CAUTION: This solvent is highly flammable.)

b. Ammonium molybdate reagent (II): Dissolve 40.1 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in approximately 500 ml distilled water. Slowly add 396 ml molybdate reagent (I). Cool and dilute to 1 liter.

c. Alcoholic sulfuric acid solution: Cautiously add 20 ml conc H_2SO_4 to 980 ml methyl alcohol with continuous mixing.

d. Dilute stannous chloride reagent (II): Mix 8 ml stannous chloride reagent (I) with 50 ml glycerol. This reagent is stable for at least 6 months.

4. PROCEDURE

4.1. Filter a portion of the sample as directed in Method A, Sec. 4.1. To a 100-ml sample containing not more than 0.2 mg PO_4-P and

free from color and turbidity, add 1 drop (0.05 ml) phenolphthalein indicator. If the sample turns pink, add strong-acid solution, dropwise, to discharge the color. If more than 5 drops are required, take a smaller sample and dilute to 100 ml with distilled water after first discharging the pink color with acid.

4.2. Add, with thorough mixing after each addition, 4.0 ml molybdate reagent (I) and 0.5 ml (10 drops) stannous chloride reagent (I). The rate of color development and the intensity of color depend on the temperature of the final solution, each 1°C increase producing about 1 percent increase in color. Hence, samples, standards, and reagents should be within 2°C of one another and at a temperature between 20° and 30°C.

4.3. After 10 min, but before 12 min, employing the same specific interval for all determinations, measure the color photometrically at 690 mμ and compare with a calibration curve, using a distilled-water blank. Light path lengths suitable for various phosphate ranges are as follows:

Approx. PO_4-P range, mg/liter	Light path, cm
0.3 to 2.0	0.5
0.1 to 1.0	2
0.01 to 0.2	10

A blank must always be run on the reagents and distilled water. Inasmuch as the color at first develops progressively and later fades, it is essential that timing be the same for samples as for standards. At least one standard should be tested with each set of samples or once each day that tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0 mg P/liter range.

4.4. Extraction: When increased sensitivity is desired or interferences need to be overcome, use an extraction method as follows: Pipet a suitable aliquot of sample into a 100-ml graduated extraction cylinder and dilute, if necessary, to 40 ml with distilled water. Add 50 ml benzene-isobutanol solvent and 15 ml molybdate reagent (II). Close at once and shake vigorously for exactly 15 sec. Any delay will increase the amount of polyphosphate hydrolysis and will increase the apparent orthophosphate value. Remove the stopper and withdraw 25 ml of separated organic layer, using a pipet and a safety aspirator. Transfer to a 50-ml volumetric flask, add 15 to 16 ml alcoholic sulfuric acid solution, swirl, add 10 drops (0.50 ml) dilute stannous chloride reagent (II), swirl, and dilute to the mark with alcoholic sulfuric acid. Mix thoroughly; after 10 min, but before 30 min, read against the blank at 625 mμ. Prepare

the blank by carrying 40 ml distilled water through the same procedure as the sample. Read the PO_4-P concentration from a calibration curve prepared by taking known phosphate standards through the same procedural steps as the samples.

5. CALCULATION

The results from the direct and the extraction procedures can be calculated by the following equation:

$$\text{mg/liter } PO_4\text{-P} = \frac{\text{mg } PO_4\text{-P} \times 1,000}{\text{ml sample}}$$

6. PRECISION AND ACCURACY

The precision, both with and without extraction, approximates ±0.0003 mg P (0.01 mg/liter) or about ±2 percent of the result, whichever is the larger numerical value.

TOTAL ORTHOPHOSPHATE

Methods A and B, described for filterable orthophosphate, can be used to determine total orthophosphate if the initial membrane filtration step in Methods A and B is omitted. Note that on samples containing large amounts of suspended sediments anomalous results may be obtained with the orthophosphate technique since it appears that a time-dependent desorption of orthophosphate takes place from the suspended particles. This produces a result that very often depends largely on the degree of agitation and mixing to which the sample is subjected during analysis.

FILTERABLE CONDENSED PHOSPHATES

1. GENERAL DISCUSSION

1.1. Principle: The polyphosphate content of the sample is operationally defined as the difference between the orthophosphate as measured by Method A or B and the phosphate found after mild acid hydrolysis. Generally, it includes the condensed phosphates such as pyro-, tripoly-, and higher molecular-weight species like hexametaphosphate. In addition, some natural waters contain organic phosphate compounds that are hydrolyzed to orthophosphate under the conditions of this test. Polyphosphates generally do not respond to the orthophosphate test but can be hydrolyzed to orthophosphate by boiling with acid.

1.2. Interference: Interferences are the same as for the orthophosphate Methods A or B, Sec. 1.2, whichever is used to complete the

the blank by carrying 40 ml distilled water through the same procedure as the sample. Read the PO_4-P concentration from a calibration curve prepared by taking known phosphate standards through the same procedural steps as the samples.

5. CALCULATION

The results from the direct and the extraction procedures can be calculated by the following equation:

$$\text{mg/liter } PO_4\text{-P} = \frac{\text{mg } PO_4\text{-P} \times 1,000}{\text{ml sample}}$$

6. PRECISION AND ACCURACY

The precision, both with and without extraction, approximates ±0.0003 mg P (0.01 mg/liter) or about ±2 percent of the result, whichever is the larger numerical value.

TOTAL ORTHOPHOSPHATE

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1.2. Interference: Interferences are the same as for the orthophosphate Methods A or B, Sec. 1.2, whichever is used to complete the

analysis. The sample should be analyzed as soon as possible after collection because polyphosphates will significantly decrease on long standing or on heating.

1.3. Minimum detectable concentration: The minimum detectable concentration will depend on whether orthophosphate Method A or B is used for the colorimetric finish after hydrolysis. The selection should be based on ease of use and sensitivity required. See Orthophosphate Methods A and B, Sec. 1.3, for details.

2. APPARATUS

In addition to the apparatus required in either Orthophosphate Method A or B, Sec. 2, an autoclave or pressure cooker capable of being operated at 15 to 20 psi may be employed to speed hydrolysis.

3. REAGENTS

3.1. Phenolphthalein indicator solution: See Orthophosphate Method A, Sec. 3.1.

3.2. Strong-acid solution: Slowly add 300 ml conc H_2SO_4 to about 600 ml distilled water. When cool add 4.0 ml conc HNO_3 and dilute to 1 liter.

3.3. Sodium hydroxide, 1 N: Dissolve 40 g NaOH in a small quantity of distilled water and dilute to 1 liter.

4. PROCEDURE

4.1. To a 100-ml portion of filtered sample (see Orthophosphate Method A, Sec. 4.1) or aliquots diluted to 100 ml, add 1 drop of phenolphthalein indicator solution. If a red color develops, add strong-acid solution dropwise to just discharge the color. Then add 1 ml in excess.

4.2. Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 ml. Alternatively, heat for 30 min in an autoclave or pressure cooker at 14 to 20 psi. Cool, neutralize to a faint pink color with sodium hydroxide solution, and restore the portions to the original 100-ml volume with distilled water.

4.3. A calibration curve should be prepared by carrying a series of standards containing orthophosphate (see Method A or B) through the hydrolysis step. Orthophosphate standards without hydrolysis cannot be used, since the salts added in hydrolysis cause an increase in the color intensity of the solution.

4.4. Determine the orthophosphate content of the treated portion by using either Orthophosphate Method A or B. This gives the sum of polyphosphate and orthophosphate in the sample.

4.5. Determine the orthophosphate in the filtered original untreated sample by the same method as used in Sec. 4.4.

5. CALCULATION

5.1. The soluble polyphosphate content of the sample may be calculated by subtracting the orthophosphate found in Sec. 4.5 from the polyphosphate plus orthophosphate value obtained in Sec. 4.4. All results are reported as mg/liter P.

6. PRECISION AND ACCURACY

The precision and accuracy are largely dependent on the method used for the determination of the phosphate contents after digesting with acid (see Sec. 6 of both Orthophosphate Methods A and B).

TOTAL PHOSPHORUS

GENERAL DISCUSSION

The total phosphorus content of the sample includes all of the orthophosphates and condensed phosphates, both soluble and insoluble, and organic and inorganic species. To release phosphate from combination with organic matter a digestion or oxidation technique is necessary. The rigor of the digestion required depends on the type of sample. In the following method, three digestion techniques are given. In order of decreasing rigor these are A. Perchloric acid digestion, B. Sulfuric acid-nitric acid digestion, and C. Persulfate digestion. It is recommended that the digestion techniques be compared for phosphorus recovery on the specific type of samples being tested and if it is found that the less tedious persulfate method gives good phosphorus recovery it should be adopted.

Following digestion the liberated orthophosphate is determined by either Orthophosphate Method A or B (pp. 78, 82).

A. PERCHLORIC ACID DIGESTION

1.1. Interferences: Interferences are the same as for Orthophosphate Method A or B (pp. 78, 82), whichever is used to complete the analysis.

1.2. Minimum detectable concentration: The minimum detectable concentration will depend on whether Orthophosphate Method A or B is used after hydrolysis.

2. APPARATUS

2.1. Hot plate: A 30 cm × 50 cm heating surface is adequate.

2.2. Safety shields.

2.3. Safety goggles.

2.4. Conical flasks, 125 ml, which have been acid washed followed by distilled water rinses.

2.5. Porcelain evaporating dishes: Acid washed and distilled water rinsed.

3. REAGENTS

3.1. Nitric acid, concentrated, reagent grade.

3.2. Perchloric acid; $HClO_4 \cdot 2H_2O$; purchased as 70 to 72 percent $HClO_4$, reagent grade.

3.3. Sodium hydroxide, 1 N: Dissolve 40 g NaOH in a small quantity of distilled water and dilute to 1 liter.

3.4. Methyl orange indicator solution: Dissolve 0.5 g methyl orange in 1 liter distilled water.

3.5. Reagents for Orthophosphate Method A or B (see pp. 78, 82).

4. PROCEDURE

4.1. CAUTION: Heated mixtures of concentrated perchloric acid and organic matter may explode violently. To avoid this hazard the following precautions should be observed: (a) Do not add perchloric acid to a hot solution that may contain organic matter. (b) Always pretreat samples containing organic matter with HNO_3 prior to addition of $HClO_4$. (c) Use a mixture of nitric and perchloric acids in starting the digestion step. (d) Avoid repeated fumings of perchloric acid in ordinary hoods. Hoods for routine operations should be all stone or asbestos-cement. Glass fume eradicators* to be attached to a water pump are convenient for occasional work with $HClO_4$.

* Such as are obtainable from G. F. Smith Chemical Co., Columbus, Ohio.

4.2. Measure out a sample containing the desired amount of phosphate (this will be determined by whether Orthophosphate Method A or B is to be used for the colorimetric finish). Acidify to methyl orange with conc HNO_3, add another 5 ml conc HNO_3, and evaporate on a steam bath or hot plate to 15 to 20 ml, covering the vessel with a watch glass when necessary to avoid loss of material by splattering. An infrared lamp may be placed over the sample to hasten the evaporation.

4.3. Transfer the evaporated sample to a 125-ml conical flask, use 5 ml conc nitric acid to rinse the evaporating dish, cool, and add 5 ml HNO_3 and 10 ml 70 to 72 percent $HClO_4$. After adding a few boiling chips, heat on a hot plate and evaporate gently until dense white fumes of $HClO_4$ just appear. If the solution is not clear at this point, cover the neck of the flask with a watch glass and keep the solution just barely boiling until it clears. A further 10 ml HNO_3 may be added to aid the oxidation.

4.4. Cool the digested solution and add one drop of aqueous phenolphthalein solution. Neutralize with 1 N NaOH solution. If necessary, filter the neutralized solution and wash the filter liberally with distilled water. Make up the filtrate to 100 ml with distilled water.

4.5. Determine the orthophosphate of the treated sample by using either Orthophosphate Method A or B.

4.6. A calibration curve should be prepared by carrying a series of standards containing orthophosphate (see Orthophosphate Method A or B) through the digestion step. Orthophosphate standards without hydrolysis cannot be used, since the salts added in digestion cause an increase in the color intensity of the solution.

B. SULFURIC ACID-NITRIC ACID DIGESTION

1.1. Interferences: Interferences are the same as for Orthophosphate Method A or B, whichever is used to complete the analysis.

1.2. Minimum detectable concentration: The minimum detectable concentration will depend on whether Orthophosphate Method A or B is used after hydrolysis.

2. APPARATUS

2.1. Digestion rack: An electrically or gas heated digestion rack with a provision for withdrawal of fumes is recommended. Digestion racks typical of those used for microkjeldahl digestions are recommended.

2.2. Microkjeldahl flasks.

3. REAGENTS

3.1. Phenolphthalein indicator solution, 0.5 percent aqueous solution.

3.2. Sulfuric acid, 1 N: Add 28 ml of concentrated H_2SO_4 slowly and cautiously to approximately 750 ml distilled water and dilute to 1 liter.

3.3. Sulfuric acid, concentrated, reagent grade.

3.4. Nitric acid, concentrated, reagent grade.

3.5. Sodium hydroxide, 2 N: Dissolve 80 g NaOH in a small quantity of distilled water and dilute to 1 liter.

4. PROCEDURE

4.1. Measure out a sample into a microkjeldahl flask containing the desired amount of phosphate (this will be determined by whether Orthophosphate Method A or B is to be used for the colorimetric finish). Add 1 ml of reagent-grade conc H_2SO_4 and 5 ml of reagent-grade conc HNO_3.

4.2. Digest sample to a volume of 1 ml and then continue the digestion until the solution becomes colorless to effect removal of HNO_3.

4.3. Cool, and add approximately 20 ml distilled water, 1 drop phenolphthalein indicator, and as much 2N NaOH solution as required to produce a faint pink tinge in the solution. Transfer the neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-ml volumetric flask and make up (following filter washings) with distilled water to 100 ml.

4.4. Determine the phosphorus present by using either Orthophosphate Method A or B, for which a separate calibration curve has been constructed by carrying through the acid digestion procedure described above.

C. PERSULFATE DIGESTION METHOD

1.1. Interferences: Interferences are the same as for Orthophosphate Method A or B, whichever is used to complete the analysis.

1.2. Minimum detectable concentration: The minimum detectable concentration will depend on whether Orthophosphate Method A or B is used after hydrolysis.

2. APPARATUS

2.1. Hot plate: A 30 cm × 50 cm heating surface is adequate.

2.2. Autoclave: An autoclave or pressure cooker capable of developing between 15 to 20 psi may be used in place of a hot plate.

3. REAGENTS

3.1. Phenolphthalein indicator solution, 0.5 percent aqueous solution.

3.2. Potassium persulfate solution: Dissolve 5 g reagent-grade $K_2S_2O_8$ in 100 ml distilled water. Prepare fresh daily.

3.3. Sulfuric acid solution, 30 percent: Carefully add 300 ml conc H_2SO_4 to approximately 600 ml distilled water and then dilute to 1 liter with distilled water.

3.4. Sodium hydroxide, 1 N: Dissolve 40 g NaOH in a small quantity of distilled water and dilute to 1 liter.

4. PROCEDURE

4.1. Take 100 ml or suitable aliquot of thoroughly mixed sample. To each 100 ml sample or aliquot diluted to 100 ml add 1 drop phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise to just discharge the color. Then add 1 ml strong acid solution and 15 ml potassium persulfate solution.

4.2. Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 ml. Alternately heat for 30 min in an autoclave or pressure cooker at 15 to 20 psi. Cool, add 1 drop phenolphthalein solution, and neutralize to a faint pink color with sodium hydroxide solution. Restore the volume to 100 ml with distilled water.

4.3. Determine the phosphorus present by using either Orthophosphate Method A or B, for which a separate calibration curve has been constructed by carrying the standards through the persulfate digestion procedure described above.

TOTAL FILTERABLE (SOLUBLE) PHOSPHATE

Total filterable (soluble) phosphate is determined in a manner similar to that for total phosphate except that the analysis is performed on a sample that has been filtered through an $0.5 \pm 0.1\ \mu$ membrane filter.

Difficulties may be encountered with highly saline samples when digestion techniques are used that drastically reduce the original sample volume because of precipitation of large quantities of salt. If it is necessary to carry out total phosphate and total filterable (soluble) phosphate on such samples it is recommended that a total filterable (soluble) phosphate analysis be conducted with the persulfate digestion technique. The total phosphate should then be determined by adding the total filterable (soluble) phosphate result to an analysis of total particulate phosphate performed on the residue remaining on the membrane filter by using the perchloric acid digestion technique. The membrane filters should be digested first in nitric acid and then in perchloric acid according to digestion Method A. This technique, besides eliminating salt precipitation problems during digestion, also makes possible the direct analysis of small amounts of particulate phosphate with a greater precision than that obtained by the subtraction of total filterable (soluble) phosphate from total phosphate (since the particles can be derived from a large sample volume).

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APPENDIX D.

BISMUTH-CATALYZED ORTHO AND POLYPHOSPHATE METHOD FOR PHOSPHATE-TREATED WATERS

1. GENERAL DISCUSSION

1.1. Principle: Orthophosphate reacts with ammonium molybdate to form a yellow molybdophosphate complex. Reduction of this yellow complex yields molybdenum blue and a considerably more intense color which is directly proportional to the amount of phosphate present. The method is free of interference from other ions which form similar complexes and yield molybdenum blue.

In the polyphosphate determination, the sample is heated in the presence of a bismuth catalyst to hydrolyze the polyphosphate completely. The ammonium molybdate reagent is present during the heating step so that the yellow molybdophosphate complex is produced as the polyphosphate is reverted. Again the yellow molybdophosphate complex is reduced to molybdenum blue to provide greater sensitivity. The same calibration curve is applicable for both methods. The presence of the bismuth catalyst in the procedure serves a dual purpose: That of shortening the heating time and that of enhancing the ultimate molybdenum blue color and thereby increasing the sensitivity of the method.

1.2. Interference: The following ions interfere in concentrations exceeding those listed:

Ion	mg/liter
Arsenic (III)	8
Germanium (III)	50*
Iron (II or III)	30
Chromate	85*
Silicate (SiO_2)	200

* Highest amount tested.

Interference from color or turbidity can be eliminated by using a specially treated portion of the sample to zero the photometer as described in Section 4.2.

1.3. Minimum detectable concentration: 200 and 30 μg/liter PO_4 with a 1-cm and 5-cm light path, respectively. These limits of detection are based on the amount required to produce a change of 1 percent transmittance when read against the zero standards.

2. APPARATUS

Colorimetric equipment: One of the following is required.

2.1. Spectrophotometer, for use at 700 mμ with a light path of 1 cm or longer.

2.2. Filter photometer, providing a light path of 1 cm or longer and equipped with a red filter exhibiting maximum transmittance in the wavelength range of 600 to 750 mμ.

3. REAGENTS

3.1. Stock phosphate solution: Dissolve in distilled water 716.5 mg potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1,000 ml; 1.00 ml = 500 μg PO_4.

3.2. Standard phosphate solution: Dilute 100.0 ml stock phosphate solution to 1,000 ml with distilled water to form a solution containing 50.0 μg PO_4 per 1.00 ml.

3.3. Nitric acid, 6N: Dilute 380 ml conc HNO_3 to 1 liter.

3.4. Acid molybdate reagent: Dissolve 400 mg bismuth nitrate pentahydrate, $Bi(NO_3)_3 \cdot 5H_2O$, in 76 ml conc nitric acid. In approximately 100 ml distilled water dissolve 2.44 g ammonium molybdate tetrahydrate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Combine the two solutions and dilute to 200 ml.

3.5. Aminonaphtholsulfonic acid reagent: In approximately 75 ml distilled water dissolve 770 mg sodium sulfite, Na_2SO_3. After complete dissolution of the Na_2SO_3, dissolve 300 mg 1-amino-2-naphthol-4-sulfonic acid, sodium salt. In another container dissolve completely 15.9 g sodium hydrogen sulfite, $NaHSO_3$, in approximately 75 ml distilled water. While stirring, pour the contents of the first container into the second. Dilute to 200 ml. Store the solution in a brown glass-stoppered bottle. The reagent is stable for approximately 2 months.

4. PROCEDURE

4.1. Preparation of calibration curve: Prepare phosphate standards in the range of 0.5 to 10.0 mg/liter by pipetting 1.00, 2.00, 5.00, 10.0, and 20.0 ml standard phosphate soltuion into 100-ml volumetric flasks and diluting to 100 ml with distilled water. Treat 10.0 ml of each solution as described under Sec. 4.2 and determine the photometer readings.

4.2. Color development: If soluble phosphate is desired, use clear supernatant or a filtered sample. If soluble plus insoluble phosphate is desired, use a portion of the well-mixed unfiltered sample. In the case of a turbid or colored sample, prepare a sample reference by simultaneously running a separate sample aliquot through the entire procedure except that 5.0 ml 6N HNO_3 is substituted for the acid molybdate reagent.

Pipet 10.0 ml clear sample, phosphate standards, and reagent blank prepared from distilled water, into clean 50-ml volumetric flasks. To each flask add 5.0 ml acid molybdate reagent. Dilute immediately to the 50-ml mark with distilled water, add 1.0 ml aminonaphtholsulfonic acid reagent to each flask, and mix well. Measure the absorbance at 700 mμ 5 minutes after the addition of the reducing agent. Null the photometer with the reagent blank for clear samples and phosphate standards, and the sample reference for turbid and colored samples.

4.3. Treatment of water samples for total (ortho- and poly-) phosphate: Determine the total phosphate by pipetting 10.0 ml sample into a clean 125-ml erlenmeyer flask. Add 5.0 ml acid molybdate solution. Swirl to mix. Place on a hot plate and heat the solution to boiling. Boil gently for 5 minutes. Cool the solution to between 16° and 29°C, then transfer quantitatively to a 50-ml volumetric flask. Dilute to the mark with distilled water and add 1.0 ml aminonaphtholsulfonic acid reducing agent. Mix thoroughly and read the absorbance 5 minutes after the addition of the reducing agent as indicated in Sec. 4.2.

5. CALCULATION

The concentration of polyphosphate is taken as the difference between the orthophosphate value and the total phosphate as determined on the filtered sample. The difference between the two total phosphate values as determined on the filtered and unfiltered portions is added to the figure obtained as soluble orthophosphate to obtain total orthophosphate. These calculations are explained in Sec. 5, Method C, page 237, 12th Edition, SMEWW.

6. PRECISION AND ACCURACY

The precision and accuracy of this method is estimated at ±0.2 mg/liter.

APPENDIX E.

TABULATION OF RESULTS

Table E-1. Sample 1, Ammonia (Nitrogen, 1.50 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	1.30	1	2411	1.48	4
2	1.57	1	2411	1.31	1
2	1.62	2	2411	1.48	3
2	1.56	3	2526	1.30	1
2	1.55	4	2726	0.50	1
4	1.21	1	2926	1.78	1
5	0.78	1	3126	1.13	1
6	0.94	1	3126	1.36	3
1112	1.64	3	3221	1.40	1
1114	1.69	1	3226	1.38	1
1123	1.38	1	3322	1.30	1
1124	1.40	1	3416	2.31	1
1124	1.42	6	3426	1.72	1
1136	1.24	1	3426	1.57	3
1211	1.48	1	3811	1.12	1
1222	13.75	1	4226	0.26	1
1314	1.11	1	4311	1.10	1
1411	1.41	1	4511	1.32	5
1524	1.62	1	4511	1.57	1
1711	1.40	2	4611	1.37	1
2144	1.01	1	4623	1.48	1
2162	1.18	1	4623	1.60	6
2222	0.48	1	4711	1.27	1
2223	1.47	1	4911	0.84	1

(Table E-1 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
5511	1.79	1	7522	1.63	1
5524	1.42	1	7526	1.38	1
5524	1.52	7	7622	1.52	1
5526	1.19	1	7813	1.50	2
5526	1.22	1	7813	1.25	4
5711	1.52	1	7813	1.60	1
5711	1.52	1	7813	1.10	3
5811	1.52	3	7813	1.60	1
5811	1.55	1	7914	1.55	1
5823	1.56	1	7922	1.12	1
6112	1.45	1	8111	1.50	3
6311	1.43	1	8111	1.60	1
6314	1.61	1	8222	1.22	1
6512	1.74	1	8222	1.40	4
6622	1.50	4	8322	1.40	1
6622	1.70	1	8613	1.39	1
6711	2.00	6	8723	1.48	1
6711	0.70	1	9325	1.80	1
6715	1.37	1	9523	1.50	4
6811	1.30	1	9713	1.55	1
6812	0.91	1	9714	1.62	1
6911	1.55	1	9714	1.52	3
7112	1.39	1	9816	1.73	1
7224	0.38	1			

Table E-2. Sample 2, Ammonia (Nitrogen, 0.80 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.71	1	2411	0.75	4
2	0.96	1	2411	0.68	1
2	0.91	2	2411	0.78	3
2	0.86	3	2526	0.65	1
2	0.88	4	2726	0.34	1
4	0.56	1	2926	0.92	1
5	0.41	1	3126	0.64	1
6	0.79	1	3126	0.61	3
1112	0.99	3	3221	0.75	1
1114	0.92	1	3226	0.66	1
1123	0.80	1	3322	1.00	1
1124	0.81	1	3416	1.26	1
1124	0.84	6	3426	0.94	1
1136	0.73	1	3426	0.89	3
1211	0.76	1	3811	0.64	1
1222	14.00	1	4226	0.19	1
1314	0.71	1	4311	0.60	1
1411	0.86	1	4511	0.67	5
1524	0.87	1	4511	0.65	1
1711	0.80	2	4611	0.75	1
2144	0.70	1	4623	0.82	6
2162	0.71	1	4623	0.76	1
2222	0.50	1	4711	0.61	1
2223	0.78	1	4911	0.75	1

(Table E-2 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
5511	0.87	1	7522	0.84	1
5524	0.93	1	7526	1.41	1
5524	0.81	7	7622	0.94	1
5526	0.79	1	7813	0.80	1
5526	0.79	1	7813	0.60	4
5711	0.80	1	7813	0.90	1
5711	0.80	1	7813	0.60	3
5811	0.85	1	7813	0.65	2
5811	0.76	3	7914	0.88	1
5823	0.76	1	7922	0.70	1
6112	0.90	1	8111	0.85	1
6311	0.89	1	8111	0.81	3
6314	0.76	1	8222	0.68	1
6512	1.00	1	8222	0.69	4
6622	1.00	1	8322	0.80	1
6622	0.95	4	8613	0.79	1
6711	1.15	6	8723	0.82	1
6711	1.00	1	9325	0.96	1
6715	0.82	1	9523	0.78	4
6811	0.72	1	9713	0.84	1
6812	0.56	1	9714	0.88	3
6911	0.86	1	9714	0.89	1
7112	0.80	1	9816	0.82	1
7224	0.18	1			

Table E-3. Sample 3, Ammonia (Nitrogen, 0.20 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.18	1	2411	0.20	4
2	0.19	1	2411	0.18	1
2	0.14	2	2411	0.21	3
2	0.26	3	2526	0.05	1
2	0.25	4	2726	0.15	1
4	0.08	1	2926	0.21	1
5	0.08	1	3126	0.10	1
6	0.28	1	3126	0.18	3
1112	0.25	3	3221	0.16	1
1114	0.25	1	3226	0.11	1
1123	0.20	1	3322	0.00	1
1124	0.22	1	3416	0.18	1
1124	0.24	6	3426	0.30	3
1136	0.15	1	3426	0.23	1
1211	0.19	1	3811	0.18	1
1222	1.80	1	4226	0.06	1
1314	0.17	1	4311	0.13	1
1411	0.20	1	4511	0.10	5
1524	0.21	1	4511	0.14	1
1711	0.19	2	4611	0.18	1
2144	0.23	1	4623	0.22	6
2162	0.28	1	4623	0.18	1
2222	0.10	1	4711	0.14	1
2223	0.21	1	4911	0.38	1

(Table E-3 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
5511	0.25	1	7522	0.16	1
5524	0.15	1	7526	1.00	1
5524	0.15	7	7622	0.25	1
5526	0.17	1	7813	0.17	2
5526	0.16	1	7813	0.16	4
5711	0.39	1	7813	0.16	1
5711	0.39	1	7813	0.18	1
5811	0.16	3	7813	0.16	3
5811	0.21	1	7914	0.18	1
5823	0.13	1	7922	0.23	1
6112	0.29	1	8111	0.20	3
6311	0.26	1	8111	0.18	1
6314	0.20	1	8222	0.22	1
6512	0.23	1	8222	0.21	4
6622	0.20	4	8322	0.15	1
6622	0.20	1	8613	0.21	1
6711	0.45	6	8723	0.13	1
6711	0.34	1	9325	0.24	1
6715	0.21	1	9523	0.37	4
6811	0.22	1	9713	0.19	1
6812	0.11	1	9714	0.40	1
6911	0.17	1	9714	0.23	3
7112	0.18	1	9816	0.25	1
7224	0.29	1			

Table E-4. Sample 1, Nitrate (Nitrogen, 0.50 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.50	4	2162	0.56	3
2	0.46	1	2222	0.07	3
2	0.47	8	2222	0.10	2
4	0.90	2	2223	0.48	2
6	0.50	2	2223	0.50	1
6	0.50	1	2411	0.51	1
1112	0.25	5	2411	0.54	2
1114	0.58	2	2526	0.50	5
1123	0.52	2	2526	0.47	2
1124	0.20	10	2726	0.50	1
1211	0.46	2	2726	0.46	2
1222	0.97	1	2926	0.50	2
1222	1.77	2	2926	0.48	1
1314	0.34	2	3126	0.59	6
1314	0.51	3	3126	0.56	2
1524	0.50	1	3221	0.50	1
1524	0.48	2	3221	0.58	2
1711	0.49	3	3226	0.47	2
1711	0.46	5	3226	0.70	1
1711	0.56	2	3322	0.40	2
1711	0.53	9	3416	0.61	1
1711	0.73	1	3426	0.37	3
2162	0.50	2	3426	0.35	2
2162	0.55	1	4226	0.52	2

(Table E-4 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
4311	0.43	2	6311	0.42	1
4311	0.46	1	6311	0.53	2
4511	0.60	1	6314	0.51	2
4511	0.50	2	6512	0.40	2
4611	0.57	3	6512	0.48	1
4611	0.64	2	6622	0.53	1
4611	0.52	1	6622	0.50	2
4623	0.51	4	6711	0.75	1
4623	0.55	2	6711	0.50	11
4711	0.90	1	6715	0.47	3
4711	1.12	2	6715	0.52	2
4911	0.51	2	6811	0.51	2
4911	0.42	1	6811	0.62	3
5511	0.47	1	6811	0.50	1
5511	0.48	2	6812	0.72	2
5524	0.48	3	6812	0.25	3
5524	0.42	2	6911	0.45	2
5711	0.50	1	7112	0.55	1
5711	0.55	2	7112	0.50	2
5811	0.50	1	7224	0.09	1
5811	0.56	2	7224	0.17	2
5823	0.50	2	7522	0.45	2
6112	0.52	1	7526	0.52	2
6112	0.48	2	7813	0.40	2

(Table E-4 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
7813	0.28	3	8613	0.30	1
7813	0.50	4	8723	0.50	1
7813	0.50	1	8723	0.46	2
7922	0.58	2	9325	0.52	2
7922	0.87	1	9523	0.49	4
8111	0.50	1	9713	0.56	1
8111	0.39	7	9713	0.55	2
8222	0.50	1	9714	0.53	1
8222	0.50	2	9714	0.55	3
8322	0.43	2	9816	0.70	2
8322	0.45	1	9816	1.00	1
8613	0.50	2			

Table E-5. Sample 2, Nitrate (Nitrogen, 5.0 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	5.00	4	2162	5.60	1
2	5.00	1	2222	0.54	3
2	4.45	8	2222	0.90	2
4	10.95	2	2223	4.80	1
6	5.23	2	2223	4.00	2
6	5.06	1	2411	4.97	1
1112	4.00	5	2411	4.95	2
1114	2.72	2	2526	3.25	5
1123	4.55	2	2526	3.15	2
1124	4.40	10	2726	4.62	1
1211	4.80	2	2726	5.54	2
1222	3.45	1	2926	5.40	2
1222	3.54	2	2926	5.11	1
1314	4.63	2	3126	7.75	6
1314	5.53	3	3126	4.77	2
1524	4.93	1	3221	5.00	1
1524	4.66	2	3221	5.00	2
1711	5.12	3	3226	4.20	2
1711	4.85	5	3226	4.50	1
1711	5.31	2	3322	6.50	2
1711	5.01	9	3416	4.57	1
1711	4.75	1	3426	4.60	3
2162	5.16	2	3426	4.50	2
2162	5.19	3	4226	4.26	2

(Table E-5 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
4311	5.02	1	6311	5.01	2
4311	4.82	2	6311	5.10	1
4511	4.75	2	6314	5.30	2
4511	6.40	1	6512	5.00	1
4611	4.90	1	6512	0.66	2
4611	6.20	2	6622	5.00	2
4611	5.50	3	6622	4.80	1
4623	5.46	2	6711	5.10	1
4623	4.98	4	6711	4.98	11
4711	10.20	1	6715	5.14	2
4711	10.38	2	6715	4.91	3
4911	4.96	1	6811	3.90	2
4911	4.96	2	6811	3.95	1
5511	5.73	2	6811	4.85	3
5511	5.20	1	6812	3.43	2
5524	4.68	3	6812	2.48	3
5524	4.05	2	6911	5.20	2
5711	5.00	2	7112	5.00	2
5711	5.00	1	7112	5.00	1
5811	4.75	1	7224	1.32	2
5811	4.76	2	7224	0.86	1
5823	4.50	2	7522	4.85	2
6112	4.25	1	7526	4.85	2
6112	4.00	2	7813	5.75	2

(Table E-5 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
7813	4.00	3	8613	3.80	1
7813	4.95	1	8723	4.90	1
7813	5.00	4	8723	4.50	2
7922	5.10	2	9325	4.90	2
7922	12.00	1	9523	5.20	4
8111	5.08	1	9713	4.80	1
8111	4.55	7	9713	4.76	2
8222	5.00	2	9714	5.10	3
8222	5.00	1	9714	4.91	1
8322	3.70	2	9816	5.90	2
8322	3.90	1	9816	5.70	1
8613	4.30	2			

Table E-6. Sample 3, Nitrate (Nitrogen, 0.05 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.05	4	2222	0.01	3
2	0.05	1	2223	0.05	2
2	0.10	8	2223	0.00	1
4	0.07	2	2411	0.12	2
6	0.05	2	2411	0.05	1
6	0.06	1	2526	0.01	2
1112	0.00	5	2526	0.01	5
1114	0.04	2	2726	0.08	2
1123	0.07	2	2726	0.09	1
1128	0.01	10	2926	0.00	2
1211	0.04	2	2926	0.00	1
1222	0.18	1	3126	0.52	6
1222	0.18	2	3126	0.06	2
1314	0.02	2	3221	0.00	1
1314	0.03	3	3221	0.00	2
1524	0.03	1	3226	0.07	2
1524	0.02	2	3226	0.28	1
1711	0.01	3	3322	0.00	2
1711	0.06	5	3416	0.02	1
1711	0.06	2	3426	0.00	2
1711	0.04	9	3426	0.00	3
1711	0.08	1	4226	0.06	2
2162	0.04	3	4311	0.10	1
2222	0.01	2	4311	0.05	2

(Table E-6 continued)

LAB. NO.	RESULTS	METHOD	LAH. NO.	RESULTS	METHOD
4511	0.04	2	6512	0.03	1
4511	0.05	1	6622	0.04	1
4611	0.11	2	6622	0.03	2
4611	0.08	3	6711	0.10	1
4623	0.06	4	6711	0.03	11
4623	0.05	2	6715	0.01	3
4711	0.50	2	6715	0.04	2
4711	0.50	1	6811	0.10	1
4911	0.05	2	6811	0.02	3
4911	0.00	1	6811	0.09	2
5511	0.05	2	6812	0.29	2
5524	0.00	2	6812	0.03	3
5524	0.00	3	6911	0.03	2
5711	0.05	2	7112	0.10	2
5711	0.05	1	7112	0.10	1
5811	0.04	2	7224	0.02	1
5811	0.09	1	7224	0.01	2
5823	0.02	2	7522	0.09	2
6112	0.01	1	7526	0.07	2
6112	0.02	2	7813	0.03	2
6311	0.05	1	7813	0.05	1
6311	0.00	2	7813	0.05	4
6314	0.05	2	7922	0.07	2
6512	0.06	2	7922	0.23	1

(Table E-6 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
8111	0.05	1	8723	0.10	1
8111	0.04	7	9325	0.05	2
8222	0.05	1	9523	0.07	4
8222	0.05	2	9713	0.08	1
8322	0.03	2	9713	0.08	2
8322	0.07	1	9714	0.08	1
8613	0.10	1	9714	0.06	3
8613	0.10	2	9816	0.40	2
8723	0.02	2	9816	0.10	1

Table E-7. Sample 1, Orthophosphate (Phosphorus, 0.10 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.25	4	1711	0.13	1
2	0.12	5	2162	0.20	6
4	0.16	1	2222	0.05	2
5	0.12	2	2223	0.13	1
6	0.10	2	2223	0.14	2
7	0.14	8	2411	0.10	1
1112	0.00	2	2411	0.10	3
1112	0.10	1	2411	0.14	2
1114	0.14	1	2526	0.10	2
1114	0.14	2	2526	0.05	1
1123	0.10	2	2714	0.13	2
1124	0.12	7	2714	0.13	1
1124	0.00	2	2726	0.33	1
1136	0.07	2	2726	0.37	2
1136	0.10	1	2926	0.21	2
1211	0.09	2	2926	0.13	1
1211	0.12	1	3126	0.11	3
1222	0.16	1	3126	0.10	1
1222	0.20	2	3221	0.13	3
1314	0.12	2	3221	0.10	2
1314	0.15	1	3226	0.25	2
1415	0.18	6	3322	0.16	1
1524	0.15	3	3416	1.00	2
1524	0.14	1	3426	0.11	3

(Table E-7 continued)

LAB. NC.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
3426	0.10	1	5711	0.13	1
3514	0.07	3	5711	0.20	2
3514	0.06	2	5811	0.15	1
4226	0.16	1	5811	0.17	2
4226	0.03	2	5823	0.10	1
4311	0.11	1	5823	0.00	2
4511	0.11	1	6112	0.15	2
4611	0.12	2	6112	0.18	3
4611	0.16	3	6311	0.20	1
4623	0.11	1	6311	0.20	3
4623	0.11	7	6314	0.40	2
4711	0.20	3	6314	0.17	1
4711	0.10	2	6512	0.15	1
4911	0.12	3	6512	0.16	2
4911	0.10	2	6622	0.12	1
5511	0.18	2	6711	0.06	1
5511	0.17	3	6711	0.06	2
5524	0.00	2	6711	0.10	7
5524	0.26	1	6715	0.09	1
5526	0.19	3	6715	0.29	2
5526	0.19	6	6811	0.13	1
5526	0.18	2	6811	0.13	2
5622	0.14	3	6812	0.15	2
5622	0.10	1	6812	0.11	5

(Table E-7 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
6911	0.00	2	8222	0.10	1
7112	0.12	3	8222	0.00	2
7112	0.11	1	8322	0.15	1
7522	0.18	1	8322	0.16	2
7526	1.43	2	8613	0.00	2
7622	0.11	1	8613	0.10	1
7813	0.10	3	8723	0.00	2
7813	0.10	6	9325	0.18	1
7813	0.13	2	9523	0.20	7
7922	0.12	1	9713	0.15	1
8111	0.10	2	9713	0.16	2
8111	0.10	5	9714	0.16	1
8111	0.10	3	9714	0.15	2
8111	0.10	1	9816	0.20	1
8111	0.09	9			

Table E-8. Sample 2, Orthophosphate (Phosphorus, 0.60 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.84	4	1711	0.66	1
2	0.65	5	2162	0.80	6
4	0.62	1	2222	0.60	2
5	0.72	2	2223	0.60	1
6	0.63	2	2223	0.66	2
7	0.59	8	2411	0.57	2
1112	0.39	2	2411	0.60	3
1112	0.59	1	2411	0.61	1
1114	0.64	2	2526	0.40	2
1114	0.69	1	2526	0.60	1
1123	0.54	2	2714	0.63	2
1124	0.62	7	2714	0.64	1
1124	1.00	2	2726	1.70	1
1136	0.54	2	2726	1.60	2
1136	0.61	1	2926	0.78	2
1211	0.61	2	2926	0.38	1
1211	0.61	1	3126	0.62	1
1222	1.60	1	3126	0.47	3
1222	1.60	2	3221	0.62	3
1314	0.60	2	3221	0.59	2
1314	0.67	1	3226	0.63	2
1415	0.82	6	3322	0.65	1
1524	0.68	3	3416	1.60	2
1524	0.62	1	3426	0.64	3

(Table E-8 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
3426	0.62	1	5711	0.73	1
3514	0.71	2	5711	0.66	2
3514	0.72	3	5811	0.57	2
4226	0.78	1	5811	0.67	1
4226	0.72	2	5823	0.14	2
4311	0.67	1	5823	0.62	1
4511	0.64	1	6112	0.69	2
4611	0.64	2	6112	1.05	3
4611	0.75	3	6311	0.84	1
4623	0.57	1	6311	0.77	3
4623	0.62	7	6314	1.05	2
4711	0.90	3	6314	0.90	1
4711	0.90	2	6512	0.78	2
4911	0.60	3	6512	0.67	1
4911	0.70	2	6622	0.62	1
5511	0.82	2	6711	0.52	2
5511	0.84	3	6711	0.49	1
5524	0.64	2	6711	0.57	7
5524	0.80	1	6715	0.69	2
5526	0.79	6	6715	0.60	1
5526	0.80	2	6811	0.63	2
5526	0.79	3	6811	0.61	1
5622	0.64	1	6812	0.68	2
5622	0.66	3	6812	0.63	5

(Table E-8 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
6911	0.60	2	8222	0.60	1
7112	0.64	1	8222	0.86	2
7112	0.62	3	8322	0.64	2
7522	0.76	1	8322	0.69	1
7526	0.60	2	8613	0.60	2
7622	0.66	1	8613	0.60	1
7813	0.60	2	8723	0.60	2
7813	0.60	6	9325	0.82	1
7813	0.60	3	9523	1.40	7
7922	0.49	1	9713	0.66	2
8111	0.60	1	9713	0.60	1
8111	0.60	5	9714	0.76	1
8111	0.58	2	9714	0.75	2
8111	0.60	3	9816	1.11	1
8111	0.62	9			

Table E-9. Sample 3, Orthophosphate (Phosphorus, 7.00 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	8.93	4	1711	7.50	1
2	7.05	5	2162	8.00	6
4	7.00	1	2222	6.75	2
5	7.00	2	2223	0.14	2
6	7.20	2	2223	7.25	1
7	7.50	8	2411	7.17	2
1112	6.90	2	2411	7.31	3
1112	7.00	1	2411	7.24	1
1114	7.14	2	2526	6.20	2
1114	7.18	1	2526	6.20	1
1123	6.82	2	2714	8.00	1
1124	7.00	7	2714	7.80	2
1124	7.20	2	2726	10.02	1
1136	7.01	1	2726	11.50	2
1136	7.13	2	2926	7.82	1
1211	7.10	2	2926	7.43	2
1211	6.85	1	3126	6.80	1
1222	8.00	1	3126	7.23	3
1222	8.00	2	3221	7.20	3
1314	7.80	2	3221	7.11	2
1314	7.10	1	3226	7.50	2
1415	8.28	6	3322	6.52	1
1524	7.33	3	3416	8.00	2
1524	7.19	1	3426	7.11	1

(Table E-9 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
3426	7.83	3	5711	7.33	1
3514	6.36	3	5711	7.30	2
3514	6.40	2	5811	6.85	2
4226	6.52	2	5811	7.00	1
4226	8.09	1	5823	1.06	1
4311	7.08	1	5823	6.94	2
4511	7.40	1	6112	7.00	2
4611	7.43	3	6112	10.00	3
4611	7.30	2	6311	7.34	3
4623	7.08	7	6311	7.45	1
4623	7.55	1	6314	8.46	1
4711	7.30	3	6314	7.30	2
4711	7.30	2	6512	8.50	2
4911	7.20	3	6512	7.30	1
4911	7.60	2	6622	7.15	1
5511	8.77	3	6711	9.02	2
5511	8.48	2	6711	9.50	7
5524	6.80	2	6711	5.73	1
5524	7.10	1	6715	8.00	1
5526	8.10	3	6715	9.17	2
5526	8.08	6	6811	6.91	2
5526	8.41	2	6811	6.91	1
5622	7.32	3	6812	7.78	5
5622	7.16	1	6812	8.04	2

(Table E-9 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
6911	7.55	2	8222	7.40	2
7112	7.20	3	8222	7.20	1
7112	7.60	1	8322	7.20	1
7522	7.34	1	8322	7.20	2
7526	0.00	2	8613	7.20	1
7622	7.50	1	8613	6.80	2
7813	7.20	3	8723	7.60	2
7813	7.20	6	9325	8.20	1
7813	7.00	2	9523	8.30	7
7922	6.72	1	9713	7.30	1
8111	7.40	3	9713	7.37	2
8111	7.15	2	9714	7.47	1
8111	7.20	5	9714	7.50	2
8111	7.10	1	9816	8.97	1
8111	7.10	9			

Table E-10. Sample 1, Polyphosphate (Phosphorus, 0.08 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.00	4	2411	0.07	1
4	0.05	1	2411	0.10	3
5	0.00	2	2526	0.20	3
6	0.28	2	2726	0.25	1
7	0.11	7	2726	0.13	2
1114	0.04	2	2926	0.03	1
1114	0.03	1	2926	0.00	2
1123	0.02	2	3126	0.09	1
1124	0.05	5	3126	0.11	3
1124	0.00	2	3221	0.16	2
1136	0.06	1	3221	0.16	3
1136	0.08	2	3226	0.00	2
1211	0.08	1	3322	0.00	1
1211	0.09	2	3416	0.10	2
1222	0.63	2	3426	0.06	3
1222	0.75	1	3426	0.04	4
1314	0.09	1	3514	0.08	2
1524	0.06	3	3514	0.09	3
1524	0.06	1	4226	0.15	1
1711	0.07	1	4311	0.04	1
2222	0.12	2	4511	0.07	1
2223	0.12	2	4611	0.16	3
2223	0.13	1	4623	0.07	5
2411	0.01	2	4623	0.07	1

(Table E-10 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
4711	0.00	3	6622	0.08	1
4711	0.10	2	6711	0.10	1
4911	0.10	3	6711	0.25	2
4911	0.04	2	6711	0.04	5
5511	0.04	3	6715	0.32	2
5524	0.00	1	6715	0.14	1
5526	0.03	2	6811	0.13	2
5526	0.02	4	6811	0.13	1
5526	0.02	3	6812	0.08	6
5622	0.06	3	6812	0.07	2
5622	0.06	1	7112	0.11	1
5711	0.03	1	7112	0.07	3
5711	0.03	2	7522	0.03	1
5811	0.07	1	7526	0.00	2
5811	0.13	2	7622	0.08	1
5823	0.00	2	7813	0.03	2
5823	0.07	1	7813	0.05	3
6112	0.18	3	7922	0.13	1
6311	0.00	3	8111	0.07	1
6311	0.02	1	8111	0.07	3
6314	0.05	1	8111	0.06	2
6314	0.00	2	8111	0.09	8
6512	0.04	1	8222	0.10	1
6512	0.01	2	8222	0.00	2

(Table E-10 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
8322	0.03	2	9713	0.26	2
8322	0.03	1	9713	0.17	1
8613	0.30	2	9714	0.06	2
8613	0.10	1	9714	0.03	1
8723	0.10	2	9816	0.00	1

Table E-11. Sample 2, Polyphosphate (Phosphorus, 0.30 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.15	4	2411	0.24	1
4	0.22	1	2411	0.30	3
5	0.15	2	2526	0.30	3
6	0.33	2	2726	0.73	2
7	0.55	7	2726	0.73	1
1114	0.26	2	2926	0.04	2
1114	0.24	1	2926	0.00	1
1123	0.21	2	3126	0.29	3
1124	0.29	5	3126	0.23	1
1124	0.00	2	3221	0.23	3
1136	0.39	2	3221	0.26	2
1136	0.35	1	3226	0.22	2
1211	0.28	2	3322	0.33	1
1211	0.33	1	3416	0.20	2
1222	3.00	2	3426	0.08	4
1222	2.80	1	3426	0.29	3
1314	0.24	1	3514	0.07	2
1524	0.26	3	3514	0.07	3
1524	0.26	1	4226	0.59	2
1711	0.25	1	4226	0.76	1
2222	0.40	2	4311	0.16	1
2223	0.18	2	4511	0.31	1
2223	0.24	1	4611	0.62	3
2411	0.31	2	4623	0.28	1

(Table E-11 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
4623	0.28	5	6512	0.11	2
4711	0.00	3	6622	0.17	1
4711	0.00	2	6711	0.35	2
4911	0.33	2	6711	0.25	1
4911	0.62	3	6711	0.10	5
5511	0.13	3	6715	0.36	1
5524	0.20	1	6715	0.32	2
5526	0.13	3	6811	0.53	2
5526	0.07	2	6811	0.53	1
5526	0.17	4	6812	0.26	6
5622	0.25	3	6812	0.26	2
5622	0.08	1	7112	0.29	3
5711	0.17	1	7112	0.31	1
5711	0.17	2	7522	0.21	1
5811	0.20	1	7526	0.00	2
5811	0.53	2	7622	0.19	1
5823	0.45	2	7813	0.30	3
5823	0.38	1	7813	0.35	2
6112	1.05	3	7922	0.67	1
6311	0.11	1	8111	0.26	1
6311	0.20	3	8111	0.28	2
6314	0.00	1	8111	0.29	3
6314	0.10	2	8111	0.28	8
6512	0.23	1	8222	0.02	2

(Table E-11 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
8222	0.13	1	9713	0.43	2
8322	0.18	1	9713	0.50	1
8322	0.24	2	9714	0.15	2
8613	0.30	2	9714	0.12	1
8613	0.20	1	9816	0.85	1
8723	0.20	2			

Table E-12. Sample 3, Polyphosphate (Phosphorus, 3.00 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	1.86	4	2411	2.48	2
4	2.07	1	2411	2.71	3
5	0.41	2	2526	1.80	3
6	2.36	2	2726	0.43	1
7	5.30	7	2726	1.10	2
1114	2.57	1	2926	0.00	1
1114	2.76	2	2926	2.43	2
1123	3.18	2	3126	3.12	3
1124	2.80	5	3126	3.50	1
1124	2.00	2	3221	2.67	2
1136	2.71	2	3221	1.90	3
1136	1.89	1	3226	2.30	2
1211	2.94	1	3322	3.26	1
1211	2.91	2	3416	3.00	2
1222	18.50	2	3426	1.04	4
1222	18.00	1	3426	3.65	3
1314	3.00	1	3514	3.40	3
1524	2.67	3	3514	3.28	2
1524	2.81	1	4226	8.12	1
1711	2.90	1	4226	6.52	2
2222	2.60	2	4311	1.70	1
2223	2.69	2	4511	2.85	1
2223	2.58	1	4611	2.16	3
2411	2.46	1	4623	2.84	5

(Table E-12 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
4623	2.00	1	6512	1.20	2
4711	2.40	2	6622	3.35	1
4711	2.50	3	6711	1.40	2
4911	2.60	2	6711	1.25	1
4911	3.00	3	6711	2.30	5
5511	1.18	3	6715	2.80	1
5524	3.10	1	6715	1.80	2
5526	1.86	3	6811	2.06	2
5526	1.03	2	6811	2.55	1
5526	2.01	4	6812	2.72	6
5622	3.51	3	6812	3.08	2
5622	1.40	1	7112	2.40	1
5711	1.80	2	7112	2.80	3
5711	1.50	1	7522	2.61	1
5811	3.10	2	7526	1.89	2
5811	3.00	1	7622	2.15	1
5823	0.00	1	7813	2.80	3
5823	0.00	2	7813	3.60	2
6112	21.00	3	7922	7.03	1
6311	2.44	3	8111	2.70	3
6311	2.55	1	8111	3.10	1
6314	3.10	2	8111	2.88	2
6314	1.87	1	8111	3.15	8
6512	2.90	1	8222	2.70	1

(Table E-12 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
8222	1.40	2	9713	3.00	1
8322	2.60	1	9713	2.92	2
8322	3.00	2	9714	1.68	1
8613	3.00	2	9714	1.70	2
8613	3.00	1	9816	1.53	1
8723	2.10	2			

Table E-13. Sample 1, Total Phosphate (Phosphorus 0.21 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	0.25	12	1524	0.33	10
2	0.22	6	1711	0.22	3
4	0.21	9	2222	0.45	2
5	0.20	4	2222	0.25	10
6	0.38	10	2223	0.16	9
6	0.38	4	2223	0.26	3
1112	0.20	9	2411	0.20	3
1112	0.13	10	2411	0.17	10
1114	0.20	9	2526	0.10	10
1114	0.26	10	2526	0.10	9
1123	0.20	10	2714	0.24	3
1123	0.20	4	2714	0.24	4
1124	0.00	10	2726	0.58	3
1124	0.00	4	2726	0.50	4
1124	0.21	11	2926	4.55	10
1124	0.19	3	3126	0.20	4
1136	0.13	10	3126	0.20	10
1136	0.14	9	3221	0.59	4
1211	0.19	9	3221	0.00	10
1222	0.52	3	3226	0.13	4
1222	0.60	4	3226	0.28	10
1314	0.14	3	3322	0.32	9
1415	0.19	13	3322	0.16	3
1524	0.29	4	3416	1.19	2

(Table E-13 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
3416	0.95	10	5526	0.26	10
3426	0.35	13	5711	0.15	3
3514	0.23	10	5711	0.14	9
3514	0.22	4	5811	0.20	9
4226	0.10	9	5811	0.24	10
4226	0.03	10	5823	0.17	9
4311	0.18	3	5823	0.00	4
4311	0.19	9	6112	0.25	4
4511	0.16	9	6112	0.38	10
4511	0.23	3	6311	0.11	9
4611	0.00	10	6314	0.19	9
4611	0.24	4	6314	0.40	10
4623	0.15	9	6512	0.21	9
4623	0.20	11	6512	0.23	10
4711	0.10	4	6622	0.24	3
4711	0.10	10	6622	0.18	9
4911	0.22	8	6711	0.38	4
4911	0.14	10	6711	0.26	9
5511	0.21	10	6711	0.18	3
5511	0.20	8	6711	0.30	10
5524	0.16	3	6715	0.29	4
5524	0.88	9	6715	0.23	3
5526	0.20	5	6811	0.24	3
5526	0.20	13	6811	0.26	10

(Table E-13 continued)

LAB. NC.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
6811	0.22	4	8111	0.20	10
6811	0.25	9	8222	0.20	9
6812	0.22	2	8222	0.00	4
6911	0.05	10	8322	0.15	9
7522	0.18	9	8322	0.18	4
7522	0.22	3	8613	0.20	4
7526	1.43	2	8613	0.30	10
7622	0.26	3	8723	0.40	10
7813	0.30	2	9325	0.19	3
7813	0.20	13	9325	0.21	1
7813	0.20	10	9523	0.23	14
7922	0.22	9	9713	0.32	9
7922	0.30	3	9713	0.43	4
8111	0.20	9	9714	0.25	2
8111	0.19	9	9714	0.25	10
8111	0.19	7	9816	0.16	3
8111	0.21	13	9816	0.24	9

Table E-14. Sample 2, Total Phosphate (Phosphorus 0.99 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	1.05	12	1524	1.00	4
2	0.99	6	1711	1.04	3
4	1.01	9	2162	1.90	13
5	0.93	4	2222	1.30	2
6	1.14	10	2222	1.08	10
6	1.13	4	2223	1.00	3
1112	1.18	10	2223	0.92	9
1112	0.93	9	2411	0.91	10
1114	1.00	10	2411	0.92	3
1114	0.90	9	2526	0.60	10
1123	1.00	10	2526	0.75	9
1123	0.40	4	2714	1.00	4
1124	0.70	10	2714	0.98	3
1124	0.30	4	2726	2.33	4
1124	0.95	11	2726	2.43	3
1124	0.92	3	2926	0.55	10
1136	0.77	9	3126	1.80	4
1136	0.95	10	3126	1.46	10
1211	0.91	9	3221	1.50	4
1222	6.50	3	3221	0.91	10
1222	7.00	4	3226	0.88	4
1314	0.55	3	3226	1.03	10
1415	0.93	13	3322	0.98	9
1524	1.03	10	3322	0.65	3

(Table E-14 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
3416	3.05	2	5526	1.07	10
3416	2.05	10	5526	1.11	5
3426	1.10	13	5711	0.90	9
3514	0.95	4	5711	0.95	3
3514	0.96	10	5811	0.94	10
4226	0.46	10	5811	0.80	9
4226	0.53	9	5823	0.90	9
4311	1.00	9	5823	0.00	4
4311	0.97	3	6112	0.95	4
4511	0.81	9	6112	1.00	10
4511	1.10	3	6311	0.65	9
4611	0.68	10	6314	0.87	9
4611	0.94	4	6314	1.50	10
4623	0.94	11	6512	1.01	10
4623	0.80	9	6512	0.95	9
4711	0.80	10	6622	0.78	9
4711	0.80	4	6622	1.05	3
4911	1.05	10	6711	1.00	4
4911	1.22	8	6711	1.30	10
5511	0.90	8	6711	0.93	3
5511	0.98	10	6711	1.00	9
5524	0.98	3	6715	1.08	3
5524	0.71	9	6715	1.10	4
5526	0.99	13	6811	1.11	4

(Table E-14 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
6811	1.16	9	8111	0.87	9
6811	1.09	10	8222	0.73	9
6811	1.14	3	8222	0.88	4
6812	0.94	2	8322	0.91	4
6911	0.62	10	8322	0.85	9
7522	1.20	3	8613	1.00	10
7522	0.87	9	8613	0.90	4
7526	0.74	2	8723	2.50	10
7622	0.89	3	9325	0.00	1
7813	1.00	10	9325	1.10	3
7813	0.90	13	9523	0.87	14
7813	1.10	2	9713	1.09	4
7922	0.90	9	9713	1.10	9
7922	1.37	3	9714	0.99	10
8111	0.95	10	9714	1.00	2
8111	0.98	13	9816	0.93	9
8111	0.78	9	9816	1.12	3
8111	0.96	7			

Table E-15. Sample 3, Total Phosphate (Phosphorus 10.23 mg/l)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
1	10.80	12	1524	10.37	10
2	10.15	6	1711	10.50	3
4	9.05	9	2162	11.00	13
5	10.70	4	2222	9.05	10
6	10.58	10	2222	9.30	2
6	10.00	4	2223	11.37	3
1112	9.53	9	2223	11.24	9
1112	9.40	10	2411	10.01	3
1114	9.93	9	2411	9.94	10
1114	10.03	10	2526	8.70	10
1123	10.00	10	2526	8.50	9
1123	10.00	4	2714	9.80	4
1124	9.50	4	2714	10.20	3
1124	10.00	10	2726	12.60	4
1124	10.60	11	2726	10.60	3
1124	10.10	3	2926	5.54	10
1136	10.16	10	3126	10.60	4
1136	8.50	9	3126	11.20	10
1211	9.80	9	3221	12.00	4
1222	19.00	3	3221	9.80	10
1222	20.40	4	3226	11.00	10
1314	8.80	3	3226	10.40	4
1415	10.34	13	3322	9.78	3
1524	10.42	4	3322	13.04	9

(Table E-15 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
3416	10.95	2	5526	10.75	5
3416	10.65	10	5526	10.08	13
3426	13.05	13	5711	9.30	3
3514	10.90	10	5711	9.30	9
3514	10.71	4	5811	9.97	10
4226	8.06	9	5811	8.54	9
4226	6.52	10	5823	0.84	9
4311	11.36	9	5823	9.36	4
4311	10.44	3	6112	7.85	4
4511	11.26	3	6112	6.75	10
4511	9.50	9	6311	8.20	9
4611	10.20	10	6314	9.60	9
4611	10.30	4	6314	10.20	10
4623	10.10	11	6512	8.90	9
4623	10.00	9	6512	10.60	10
4711	10.80	4	6622	9.60	9
4711	10.60	10	6622	11.30	3
4911	10.20	10	6711	11.50	10
4911	10.20	8	6711	9.20	9
5511	10.19	10	6711	11.00	4
5511	10.35	8	6711	5.10	3
5524	11.30	3	6715	10.80	3
5524	10.70	9	6715	9.40	4
5526	9.67	10	6811	8.97	9

(Table E-15 continued)

LAB. NO.	RESULTS	METHOD	LAB. NO.	RESULTS	METHOD
6811	8.81	3	8111	10.00	10
6811	9.29	10	8222	9.90	9
6811	9.46	4	8222	8.80	4
6812	11.16	2	8322	10.30	4
6911	9.84	10	8322	9.90	9
7522	9.39	9	8613	10.00	10
7522	9.95	3	8613	10.00	4
7526	8.03	2	8723	11.80	10
7622	10.00	3	9325	11.00	1
7813	10.30	10	9325	10.00	3
7813	10.20	2	9523	9.70	14
7813	10.20	13	9713	10.30	9
7922	12.00	9	9713	10.30	4
7922	13.33	3	9714	10.00	10
8111	10.00	9	9714	10.40	2
8111	10.50	9	9816	10.52	9
8111	10.35	13	9816	10.60	3
8111	9.90	7			

APPENDIX F.

GLOSSARY OF STATISTICAL TERMS

A glossary of statistical terms defined as they are used in this report is presented to ensure uniformity of understanding.

Arithmetic mean — The sum of the sample results divided by the number of results in the sample. Let X_i ($i = 1, 2, \ldots, n$) denote the i^{th} result in a sample of n results. The arithmetic mean denoted $\overline{X}$ is given by $\overline{X} = \sum_{i=1}^{n} \frac{X_i}{n}$.

Median — Halfway point in the results when they have been arranged in order of magnitude (the middle result of an odd number of results, or the average of the middle two for an even number).

Accuracy — The correctness of a measurement, or the degree of correspondence between the results and the true value (actual amount added).

Measures of accuracy — Measures that relate to the difference between the mean of the results and the true value when the latter is known or assumed. The following measures apply:

Mean error – The average difference with regard to sign between the results and the true value. Equivalently, the difference between the mean of the results and the true value (T.V.).

$$\text{Mean error} = \overline{X} - \text{T.V.}$$

Relative error – The absolute value of the mean error expressed as a percentage of the true value.

$$\text{Relative error} = \frac{|\overline{X} - \text{T.V.}|}{\text{T.V.}} \times 100$$

Precision — The reproducibility of sample results or the degree of agreement among the results.

Measures of precision

Measures of the variation among the sample results themselves, i.e., the spread or dispersion of the results without regard to the true value. The following measures apply:

Sample variance – Sum of squared deviations of the sample results from their mean divided by one less than the number of results in the sample. The sample variance denoted s^2 is given by

$$s^2 = \frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n - 1}$$

where n is the number of results.

Sample standard deviation – Square root of the sample variance.

$$s = \sqrt{\frac{\sum_{i=1}^{n} (X_i - \bar{X})^2}{n - 1}}$$

Relative standard deviation (coefficient of variation) – Sample standard deviation expressed as a percentage of the mean.

$$\text{Rel. Std. Dev.} = \frac{s}{\bar{X}} \times 100$$

Range – The difference between the largest and smallest results in the sample.

Confidence limits – Limits within which the true mean, μ, of the population (the theoretically infinite number of possible replications of the analysis) will lie with probability equal to $1 - \alpha$, where α is the probability that the limits do not contain the true mean. The upper and lower $1 - \alpha$ confidence limits are given by

$$\text{Confidence limits} = \bar{X} \pm t_{\alpha/2} s/\sqrt{n}$$

where $\overline{X}$ and s are the sample mean and standard deviation, $t_{\alpha/2}$ is the upper $\alpha/2$ point of "Student's" t-distribution, and n is the number of results in the sample used to compute $\overline{X}$.

Tolerance limits – Limits within which one can state with probability γ that at least a proportion P of the entire population will lie. The upper and lower tolerance limits are given by

$$\text{Tolerance limits} = \overline{X} \pm Ks,$$

where K is the factor for two-sided tolerance limits for normal populations.[1] The value of K depends upon the chosen values of γ and P.

REFERENCES

1. Natrella, M. G. Experimental Statistics. National Bureau of Standards, 1963, p. T-10.

APPENDIX G.

TESTS FOR NORMALITY AND REJECTION OF OUTLIERS

Test for normality

The Kolmogorov-Smirnov goodness-of-fit test was used to determine whether the observations reported could reasonably be thought to have come from a normal distribution.[1]

Briefly, the test involves computing the observed cumulative frequency distribution (the percent of values less than or equal to each value in the distribution) and comparing it with the theoretical normal cumulative frequency distribution. The point at which the two distributions, theoretical and observed, show greatest divergence is determined. Reference of the value of the divergence to a table of critical values for the Kolmogorov-Smirnov goodness-of-fit test indicates whether such a large divergence is likely on the basis of chance. If such a large divergence is not likely, the distribution is designated as nonnormal; otherwise the distribution is designated as normal.

Tests for rejection of outliers

1. If the distribution is designated as nonnormal, the suspected outlier (the farthest value from the mean) is rejected only if the distance between it and the mean is greater than three standard deviations; otherwise the suspected outlier is accepted.

2. If the distribution is designated as normal and the sample size is less than or equal to 30, the suspected outlier, the farthest value from the mean, is tested for rejection by a method developed by Dixon.[2] Briefly, this test involves computing a ratio that compares the distance of the suspected value being tested from its neighbors with the range of all, or almost all, of the observations (depending on the total number of suspected values in the sample). Reference of the ratio to a table of critical values for test ratios for gross errors indicates whether such a large ratio is likely on the basis of chance. If the ratio is greater than or equal to the critical value, the probability that the suspected outlier is from the sample distribution is small; hence, the outlier is rejected. If the ratio is less than the critical value, the suspected outlier probably came from the sample distribution; hence, the suspected outlier is accepted.

3. If the distribution is designated as normal, and the sample size is greater than 30, the suspected outlier is tested for rejection by a method developed by Santner.[3] This method employs the statistic, $\frac{\overline{X} - X_o}{s}$, where $\overline{X}$ is the sample mean, X_o is the suspected outlier (the

farthest value from the mean), and s is the sample standard deviation. This statistic is compared with a table of critical values to determine whether its value is larger than would be expected on the basis of chance. If the statistic is greater than or equal to the critical value, the suspected outlier is rejected; otherwise, the suspected outlier is accepted.

Application of tests for normality and for rejection of outliers to ARS studies

The test for normality and the subsequent test for rejection of outliers are applied to the observed data in two ways: first, to each method for a given substance at a given concentration; then to a given substance at a given concentration regardless of method. In either case, it is first necessary to determine whether the original distribution is normal or nonnormal. If the original distribution is designated as nonnormal, method 1 is used to test for rejection of the suspected outlier farthest from the mean. If the suspected outlier is not rejected, no further tests for normality or rejection of outliers are made, and the distribution is designated as nonnormal. On the other hand, if the suspected outlier is rejected, the new distribution, which excludes the rejected observation, is then tested for normality. If the new distribution is nonnormal, the next suspected outlier is tested for rejection by method 1. This cycle of testing for normality and testing for rejection of outliers continues until a suspected value is not rejected or the test for normality designates the distribution as normal. If the distribution is designated as normal, subsequent tests for rejection of outliers made by method 2 or 3 are the same as if the original distribution had been normal. This case is discussed next.

If the original distribution is designated as normal or a new distribution that was originally nonnormal is designated as normal after the rejection of one or more outliers, and if the number of observations is not greater than 30, then method 2 is used to test for rejection the suspected outlier farthest from the mean. If the suspected outlier is not rejected, no further tests are made, and the distribution is designated as normal. If the suspected outlier is rejected, then the suspected outlier farthest from the mean of the new distribution is tested for rejection, and so on until the suspected value of a new distribution is not rejected; when this occurs, no further tests are made, and the final distribution is designated as normal. On the other hand, if the number of observations in the original distribution is greater than 30, method 3 is used to test the suspected outlier for rejection. If the suspected outlier is not rejected, no further tests are made, and the distribution is designated as normal. If the suspected outlier is rejected, then the suspected outlier farthest from the mean of the new distribution, which excludes the rejected value, is tested for rejection. Testing for outliers continues by this method until a suspected outlier is not rejected or the number of

observations is no longer greater than 30, in which case, method 2 is used for testing for rejection of any remaining suspected outliers.

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1. Siegel, S. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill Book Co., Inc. New York, N.Y., 1956, pp. 47-51.

2. Dixon, W. J., Ratios Involving Extreme Values. Ann. Math. Stat. 22: 68-78, 1951.

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APPENDIX H.

COMPARISON OF METHODS FOR STATISTICALLY SIGNIFICANT DIFFERENCES IN PRECISION AND ACCURACY

The methods are compared in two ways with respect to precision and accuracy. In the first case, two methods are compared at a given concentration with respect to precision and to accuracy. The unknown variances, σ_1^2 and σ_2^2 (estimated by the sample variances, s_1^2 and s_2^2), of the two methods are first compared by the F-test[1] to determine whether there is a significant difference in the precision of the two methods. The unknown means, μ_1 and μ_2 (estimated by the sample means, $\overline{X}_1$ and $\overline{X}_2$), of the two methods are then compared by the t-test[2] to determine whether there is a significant difference in the accuracy of the two methods. The t-test employed is based on the result of the F-test. These two tests of hypotheses will produce one of the following results.

Outcome 1: $\sigma_1^2 = \sigma_2^2$, $\mu_1 = \mu_2$

Outcome 2: $\sigma_1^2 = \sigma_2^2$, $\mu_1 \neq \mu_2$

Outcome 3: $\sigma_1^2 \neq \sigma_2^2$, $\mu_1 = \mu_2$

Outcome 4: $\sigma_1^2 \neq \sigma_2^2$, $\mu_1 \neq \mu_2$

In outcome 1, we conclude that the sample results do not indicate that a significant difference in either precision or accuracy exists between the two methods.

In outcome 2, we conclude that there is no indication of a significant difference in precision between the two methods, but there is a signifcant difference in the accuracy of the two methods; specifically, the method whose sample mean is closer to the true value is deemed the more accurate. In outcome 3, we conclude that there is no indication of a significant difference in the accuracy of the two methods, but the method with the smaller sample variance is the more precise.

In outcome 4, we conclude that there is a significant difference in the precision and in the accuracy of the two methods. The method with the smaller sample variance is the more precise, and the method whose sample mean is closer to the true value is the more accurate.

In the second case, more than two methods are compared at a given concentration with respect to precision and accuracy. Bartlett's Test[3] is used first to test the hypothesis of equality of the unknown variances, σ_i^2, of the methods in order to compare the precision of the methods. If we conclude that the precision is the same, the Analysis of Variance[4] is then used to test whether a significant difference exists among the means, μ_i, in order to compare the accuracy of the methods. If there is a significant difference among the means, Duncan's Multiple Range Test[5, 6] is used to determine which method means differ significantly. If the precision is not the same, then the Kruskal-Wallis One-way Analysis of Variance by Ranks[7] is used to determine whether a significant difference exists among the means in order to compare the accuracy of the methods.

Once again, there are basically four possible outcomes for the above tests of hypotheses.

Outcome 1: all σ_i^2 are equal, all μ_i are equal

Outcome 2: all σ_i^2 are equal, not all μ_i are equal

Outcome 3: not all σ_i^2 are equal, all μ_i are equal

Outcome 4: not all σ_i^2 are equal, not all μ_i are equal

In outcome 1, we conclude that the sample results do not indicate a significant difference in either the precision or the accuracy of the methods.

In outcome 2, we conclude that there is no indication of a significant difference in the precision of the methods; however, at least one method does differ significantly from the rest with respect to accuracy, and Duncan's Multiple Range Test indicates which methods differ. For example, in comparing four methods, we might conclude $\mu_1 = \mu_2$ and $\mu_3 = \mu_4$, but μ_1 and μ_2 differ significantly from μ_3 and μ_4; or we might conclude that $\mu_1 = \mu_2 = \mu_3$ but μ_4 differs significantly from μ_1, μ_2, and μ_3.

In outcome 3, we conclude that there is no indication of a significant difference in the accuracy of the methods, but at least one method differs significantly from the rest with respect to precision.

In outcome 4, we conclude that the methods differ significantly with respect to both precision and accuracy.

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5. Ibid., pp. 31-33.

6. Kramer, C. Extension of Multiple Range Tests to Group Means with Unequal Numbers of Replications. Biometrics 12: 307-310, 1956.

7. Siegel, S. Nonparametric Statistics. McGraw-Hill. New York, N.Y., 1956, pp. 184-194.

APPENDIX I.

ANALYTICAL REFERENCE SERVICE MEMBERSHIP

STATE AGENCIES

Alabama State Department of Public Health, Montgomery
Alabama Water Improvement Commission, Montgomery
Arizona State Department of Health, Phoenix
Arkansas Pollution Control Commission, Little Rock
Arkansas State Department of Health, Little Rock
California Department of Water Resources, Sacramento
California State Department of Public Health, Los Angeles
California State Department of Public Health, Air and Industrial Hygiene Laboratory, Berkeley
California State Department of Public Health, Sanitation and Radiation Laboratory, Berkeley
Colorado Department of Public Health, Denver
Connecticut State Department of Health, Hartford
Delaware Water and Air Resources Commission, Dover
District of Columbia Department of Public Health, Washington, D.C.
District of Columbia Department of Sanitary Engineering, Washington, D. C.
Florida Department of Agriculture, Tallahassee
Florida State Board of Health, Jacksonville
Florida State Board of Health, Pensacola
Florida State Board of Health, Winter Haven
Hawaii State Department of Health, Laboratories Branch, Honolulu
Hawaii State Department of Health, Occupational and Radiological Health Section, Honolulu
Idaho Department of Health, Boise
Illinois Department of Public Health, Springfield
Illinois State Water Survey, Urbana
Illinois State Water Survey, Peoria
Indiana State Board of Health, Indianapolis
Iowa State Hygienic Laboratory, Des Moines
Iowa State Hygienic Laboratory, Iowa City
Kentucky State Department of Health, Division of Laboratory Services, Frankfort
Kentucky State Department of Health, Radiological Health Program, Frankfort
Lawrence Experiment Station, Massachusetts
Louisiana State Department of Health, New Orleans
Los Angeles County Flood Control District, California
Maryland State Department of Health, Bureau of Environmental Chemistry, Baltimore
Maryland State Department of Health, Bureau of Laboratories, Baltimore
Maryland State Department of Water Resources, Annapolis

Massachusetts Department of Public Health, Amherst
Massachusetts Department of Public Health, Boston
Michigan Department of Conservation, Lansing
Michigan Department of Public Health, Lansing
Minnesota Department of Agriculture, St. Paul
Minnesota Department of Health, Minneapolis
Missouri Department of Health, Jefferson City
Montana Bureau of Mines and Geology, Butte
Montana Health Department, Helena
Nebraska State Department of Health, Lincoln
Nevada State Department of Health, Reno
Nevada State Department of Health and Welfare, Las Vegas
New Hampshire State Department of Health, Concord
New Hampshire Water Supply and Pollution Control Commission, Concord
New Jersey State Department of Health, Trenton
New Mexico Department of Public Health, Santa Fe
New York State Conservation Department, Avon
New York State Conservation Department, Ronkonkoma
New York State Department of Health, Division of Air Resources, Albany
New York State Department of Health, Division of Laboratories and Research, Albany
New York State Department of Health, Syracuse
New York State Department of Labor, New York City
North Carolina Department of Water and Air Resources, Raleigh
North Dakota State Department of Health, Bismarck
North Jersey District Water Supply Commission, Wanaque
Ohio Department of Agriculture, Reynoldsburg
Ohio State Department of Health, Columbus
Oklahoma State Health Department, Oklahoma City
Oregon State Board of Health, Portland
Pennsylvania Department of Agriculture, Harrisburg
Pennsylvania Department of Health, Division of Air Pollution Control, Harrisburg
Pennsylvania Department of Health, Water Quality Section, Harrisburg
Puerto Rico Institute of Health Laboratories, Hato Rey
Puerto Rico Aqueduct and Sewer Authority, San Juan
Rhode Island State Department of Health, Providence
South Carolina Pollution Control Authority, Columbia
South Dakota Department of Health, Pierre
Tennessee Department of Public Health, Nashville
Tennessee Stream Pollution Control Authority, Nashville
Texas State Department of Health, Austin
Utah State Department of Health, Salt Lake City
Vermont State Department of Health, Barre
Vermont State Department of Health, Burlington
Virginia State Department of Health, Bureau of Industrial Hygiene, Richmond

Virginia State Department of Health, Bureau of Laboratories, Richmond
Virginia State Water Control Board, Richmond
Washington State Department of Health, Seattle
Washington State Food and Drug Laboratory, Seattle
West Virginia Department of Natural Resources, Charleston
Wisconsin Department of Agriculture, Madison

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Albuquerque Department of Environmental Health, Air Management Division, New Mexico
Albuquerque Department of Environmental Health, Food and Institutional Division, New Mexico
Baltimore City Health Department, Maryland
Bay Area Air Pollution Control District, San Francisco, California
Beaumont Health Department, Texas
Central Water Filtration Plant, Chicago, Illinois
City of Amarillo, Water Reclamation Department, Texas
City of Charlotte, Water Department, North Carolina
City of Cincinnati, Division of Water Pollution Control, Ohio
City of Durham, Department of Water Resources, North Carolina
City of Erie, Bureau of Water, Pennsylvania
City of Long Beach, Water Department, California
City of Miami, Alexander Orr, Jr. Water Treatment Plant, Florida
City of Newburgh, Water Department, New York
City of New York, Food and Drug Laboratory, New York
City of Niagara Falls, Division of Water Laboratories, New York
City of Philadelphia, Office of the Medical Examiner, Pennsylvania
City of San Jose, Health Department, California
City of Seattle, Water Department, Washington
City of Toledo, Division of Pollution Control, Ohio
City of Yonkers, Bureau of Water, New York
County of Fresno, Department of Public Health, California
County of Los Angeles, Air Pollution Control District, California
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Department of Public Health, Environmental Health Laboratory, Philadelphia, Pennsylvania
Department of Public Health, Public Health Laboratory, Philadelphia, Pennsylvania
Department of Public Works and Utilities, Flint, Michigan
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East Bay Municipal Utility District, Oakland, California
Easterly Pollution Control Center, Cleveland, Ohio
Erie County Health Laboratory, Buffalo, New York
Hackensack Water Company, New Milford, New Jersey

Houston City Health Department, Texas
Los Angeles Department of Public Works, Playa Del Rey, California
Louisville Water Company, Kentucky
Metropolitan Denver Sewage Disposal District No. 1, Commerce City, Colorado
Metropolitan St. Louis Sewer District, Missouri
Metropolitan Sanitary District of Greater Chicago, Illinois
Metropolitan Utilities District, Omaha, Nebraska
Metropolitan Water District of Southern California, La Verne
Minneapolis Water Department, Minnesota
Monroe County Health Department, Rochester, New York
Nassau County Department of Health, Hempstead, New York
Nassau County Department of Health, Mineola, New York
New York City Health Department, New York
Orange County Air Pollution Control District, Anaheim, California
Philadelphia Water Department, Pennsylvania
Philadelphia Water Department, Belmont Laboratory, Pennsylvania
Philadelphia Water Department, Torresdale Laboratory, Pennsylvania
Riverside County Air Pollution Control District, California
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San Diego County Department of Public Health, California

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DHEW, PHS, Bureau of Water Hygiene, Bethesda, Maryland
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DHEW, PHS, Northeast Water Hygiene Laboratory, Narragansett, Rhode Island
DHEW, PHS, Northeastern Radiological Health Laboratory, Winchester, Massachusetts
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First U.S. Army Medical Laboratory, Fort George G. Meade, Maryland
Fourth U.S. Army Medical Laboratory, Fort Sam Houston, Texas
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Regional Environmental Health Laboratory (SGHK), Kelly AFB, Texas
Reynolds Electrical and Engineering Company, Inc., Las Vegas, Nevada
San Francisco Bay Naval Shipyard, Vallejo, California
Sixth U.S. Army Medical Laboratory, Sausalito, California
Tennessee Valley Authority, Chattanooga
Tennessee Valley Authority, Muscle Shoals, Alabama

Tenth U.S. Army Medical Laboratory, APO, New York
U.S. Army Environmental Hygiene Agency, Edgewood Arsenal, Maryland
USDA, Soils Laboratory, Beltsville, Maryland
USDI, FWPCA, AWTR Research Activities, Pomona, California
USDI, FWPCA, Alaska Water Laboratory, College
USDI, FWPCA, Analytical Quality Control, Cincinnati, Ohio
USDI, FWPCA, Chicago Program Office, Illinois
USDI, FWPCA, National Field Investigations Center, Cincinnati, Ohio
USDI, FWPCA, North Atlantic Water Quality Management Office, Edison, New Jersey
USDI, FWPCA, Ohio River Basin Project, Evansville, Indiana
USDI, FWPCA, Ohio River Basin Project, Wheeling, West Virginia
USDI, FWPCA, Robert S. Kerr Water Research Center, Ada, Oklahoma
USDI, FWPCA, Waste Identification and Analysis, Cincinnati, Ohio
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American Public Health Association, Riverside, California
American Water Works Association, New York, New York

Anaconda Company, Grants, New Mexico
ARMCO Steel Corporation, Middletown, Ohio
Battelle Memorial Institute, Columbus, Ohio
Bethlehem Steel Corporation, Bethlehem, Pennsylvania
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Borg-Warner Corporation, Des Plaines, Illinois
Bowser-Morner Testing Laboratories, Incorporated, Dayton, Ohio
Brown and Caldwell Laboratories, San Francisco, California
Calgon Corporation, Pittsburgh, Pennsylvania
California Water Service Company, San Jose, California
Carnation Research Laboratories, Van Nuys, California
Chrysler Corporation, Detroit, Michigan
Culligan, Incorporated, Northbrook, Illinois
Cyrus Wm. Rice and Company, Pittsburgh, Pennsylvania
Dow Chemical Company, Midland, Michigan
Emery Industries, Incorporated, Cincinnati, Ohio
Fairbanks, Morse and Company Research Center, Beloit, Wisconsin
Goodyear Atomic Corporation, Piketon, Ohio
H. C. Nutting Company, Cincinnati, Ohio
Hach Chemical Company, Ames, Iowa
Hammond-Montel, Incorporated, Elmhurst, New York
Havens-Emerson, East Patterson, New Jersey
Hill Top Research, Incorporated, Miamiville, Ohio
Holzmacher, McLendon and Murrell, Melville, New York
Hydro Research Laboratories, Pontiac, Michigan
Industrial Chemicals, Incorporated, South Bend, Indiana
INFILCO, General American Transportation Corporation, Tucson, Arizona
Ionac Chemical Company, Birmingham, New Jersey
Ionics, Incorporated, Watertown, Massachusetts
Isotopes - A Teledyne Company, Sandusky, Ohio
Isotopes, Incorporated, Palo Alto, California
Johns-Manville Research and Engineering Center, Manville, New Jersey
Kem-Tech Laboratories, Incorporated, Baton Rouge, Louisiana
Kennecott Copper Corporation, Salt Lake City, Utah
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Pacific Gas and Electric Company, Emeryville, California
Pan American World Airways, Patrick AFB, Florida
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Ray W. Hawksley Company, Incorporated, Richmond, California
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United States Pipe and Foundry Company, Birmingham, Alabama
W. R. Grace and Company, Lake Zurich, Illinois
Wastewater Analysis Corporation, Lincoln Park, Michigan
Water Pollution Control Federation, Washington, D. C.
Water Service Laboratories, Incorporated, New York, New York
Williams and Works, Grand Rapids, Michigan
Xerox Corporation, Webster, New York
York Research Corporation, Stamford, Connecticut